```
=> fil .bec
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.06       0.27

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 11:33:30 ON 14 JUN 2005
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

11 FILES IN THE FILE LIST

=> s hepadnavir? or whv or hbv or hepatitis b virus
FILE 'MEDLINE'
           751 HEPADNAVIR?
           308 WHV
         13522 HBV
        129928 HEPATITIS
        615165 B
        394609 VIRUS
         21124 HEPATITIS B VIRUS
                 (HEPATITIS(W)B(W)VIRUS)
L1       24665 HEPADNAVIR? OR WHV OR HBV OR HEPATITIS B VIRUS

FILE 'SCISEARCH'
           683 HEPADNAVIR?
           274 WHV
         11259 HBV
         95414 HEPATITIS
       1281469 B
        343776 VIRUS
         16353 HEPATITIS B VIRUS
                 (HEPATITIS(W)B(W)VIRUS)
L2       20666 HEPADNAVIR? OR WHV OR HBV OR HEPATITIS B VIRUS

FILE 'LIFESCI'
           446 HEPADNAVIR?
           202 WHV
          4964 HBV
         23391 "HEPATITIS"
        202723 "B"
        194146 "VIRUS"
          9236 HEPATITIS B VIRUS
                 ("HEPATITIS"(W)"B"(W)"VIRUS")
L3        9716 HEPADNAVIR? OR WHV OR HBV OR HEPATITIS B VIRUS

FILE 'BIOTECHDS'
            36 HEPADNAVIR?
            14 WHV
           687 HBV
          5337 HEPATITIS
         58556 B
         49264 VIRUS
          2324 HEPATITIS B VIRUS
                 (HEPATITIS(W)B(W)VIRUS)
L4        2385 HEPADNAVIR? OR WHV OR HBV OR HEPATITIS B VIRUS

FILE 'BIOSIS'
         19407 HEPADNAVIR?
           341 WHV
         13478 HBV
        108084 HEPATITIS
        723949 B
        511726 VIRUS
         27363 HEPATITIS B VIRUS
```

```
                (HEPATITIS(W)B(W)VIRUS)
L5       31268 HEPADNAVIR? OR WHV OR HBV OR HEPATITIS B VIRUS

FILE 'EMBASE'
           618 HEPADNAVIR?
           279 WHV
         11538 HBV
        101459 "HEPATITIS"
        699489 "B"
        437675 "VIRUS"
         21908 HEPATITIS B VIRUS
                ("HEPATITIS"(W)"B"(W)"VIRUS")
L6       24170 HEPADNAVIR? OR WHV OR HBV OR HEPATITIS B VIRUS

FILE 'HCAPLUS'
           765 HEPADNAVIR?
           291 WHV
          7805 HBV
         47682 HEPATITIS
       1512208 B
        320479 VIRUS
         12728 HEPATITIS B VIRUS
                (HEPATITIS(W)B(W)VIRUS)
L7       14049 HEPADNAVIR? OR WHV OR HBV OR HEPATITIS B VIRUS

FILE 'NTIS'
             1 HEPADNAVIR?
             8 WHV
            88 HBV
          1232 HEPATITIS
         67318 B
          7551 VIRUS
           126 HEPATITIS B VIRUS
                (HEPATITIS(W)B(W)VIRUS)
L8          166 HEPADNAVIR? OR WHV OR HBV OR HEPATITIS B VIRUS

FILE 'ESBIOBASE'
           332 HEPADNAVIR?
           139 WHV
          4243 HBV
         23520 HEPATITIS
        336386 B
        102747 VIRUS
          5093 HEPATITIS B VIRUS
                (HEPATITIS(W)B(W)VIRUS)
L9         6182 HEPADNAVIR? OR WHV OR HBV OR HEPATITIS B VIRUS

FILE 'BIOTECHNO'
           462 HEPADNAVIR?
           210 WHV
          5147 HBV
         27744 HEPATITIS
        228519 B
        178689 VIRUS
          8427 HEPATITIS B VIRUS
                (HEPATITIS(W)B(W)VIRUS)
L10        9206 HEPADNAVIR? OR WHV OR HBV OR HEPATITIS B VIRUS

FILE 'WPIDS'
           150 HEPADNAVIR?
            15 WHV
           936 HBV
         12857 HEPATITIS
       1199686 B
```

```
         39522 VIRUS
          1691 HEPATITIS B VIRUS
                 (HEPATITIS(W)B(W)VIRUS)
L11       2114 HEPADNAVIR? OR WHV OR HBV OR HEPATITIS B VIRUS

TOTAL FOR ALL FILES
L12     144587 HEPADNAVIR? OR WHV OR HBV OR HEPATITIS B VIRUS

=> s src or fyn or yes or lyn or blk or fgr or hck
FILE 'MEDLINE'
         15564 SRC
          1562 FYN
          3804 YES
          1088 LYN
           137 BLK
           491 FGR
           380 HCK
L13       20041 SRC OR FYN OR YES OR LYN OR BLK OR FGR OR HCK

FILE 'SCISEARCH'
         14911 SRC
          1650 FYN
          3460 YES
          1359 LYN
           133 BLK
           472 FGR
           453 HCK
L14       19817 SRC OR FYN OR YES OR LYN OR BLK OR FGR OR HCK

FILE 'LIFESCI'
          5735 SRC
           855 FYN
           479 YES
           602 LYN
            89 BLK
           159 FGR
           197 HCK
L15        6716 SRC OR FYN OR YES OR LYN OR BLK OR FGR OR HCK

FILE 'BIOTECHDS'
           306 SRC
            44 FYN
            55 YES
            28 LYN
            14 BLK
            22 FGR
            20 HCK
L16         388 SRC OR FYN OR YES OR LYN OR BLK OR FGR OR HCK

FILE 'BIOSIS'
         14939 SRC
          1762 FYN
          2072 YES
          1245 LYN
           173 BLK
           485 FGR
           427 HCK
L17       18385 SRC OR FYN OR YES OR LYN OR BLK OR FGR OR HCK

FILE 'EMBASE'
         11510 SRC
          1417 FYN
          2740 YES
           993 LYN
```

```
           124 BLK
           409 FGR
           332 HCK
L18      15222 SRC OR FYN OR YES OR LYN OR BLK OR FGR OR HCK

FILE 'HCAPLUS'
         15392 SRC
          1656 FYN
          2548 YES
          1394 LYN
           256 BLK
           716 FGR
           504 HCK
L19      19312 SRC OR FYN OR YES OR LYN OR BLK OR FGR OR HCK

FILE 'NTIS'
          2025 SRC
            20 FYN
           339 YES
            13 LYN
             9 BLK
            46 FGR
             3 HCK
L20       2442 SRC OR FYN OR YES OR LYN OR BLK OR FGR OR HCK

FILE 'ESBIOBASE'
          8008 SRC
          1062 FYN
           836 YES
           754 LYN
            82 BLK
           207 FGR
           258 HCK
L21       9521 SRC OR FYN OR YES OR LYN OR BLK OR FGR OR HCK

FILE 'BIOTECHNO'
          7046 SRC
           833 FYN
           388 YES
           605 LYN
            76 BLK
           199 FGR
           218 HCK
L22       7876 SRC OR FYN OR YES OR LYN OR BLK OR FGR OR HCK

FILE 'WPIDS'
           951 SRC
           137 FYN
          1160 YES
            89 LYN
           107 BLK
            89 FGR
            90 HCK
L23       2199 SRC OR FYN OR YES OR LYN OR BLK OR FGR OR HCK

TOTAL FOR ALL FILES
L24     121919 SRC OR FYN OR YES OR LYN OR BLK OR FGR OR HCK

=> s l12(15a)l24
FILE 'MEDLINE'
L25          7 L1 (15A)L13

FILE 'SCISEARCH'
L26          6 L2 (15A)L14
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FILE 'LIFESCI'
L27          6 L3 (15A)L15

FILE 'BIOTECHDS'
L28          1 L4 (15A)L16

FILE 'BIOSIS'
L29          7 L5 (15A)L17

FILE 'EMBASE'
L30          7 L6 (15A)L18

FILE 'HCAPLUS'
L31         14 L7 (15A)L19

FILE 'NTIS'
L32          0 L8 (15A)L20

FILE 'ESBIOBASE'
L33          6 L9 (15A)L21

FILE 'BIOTECHNO'
L34          5 L10(15A)L22

FILE 'WPIDS'
L35          1 L11(15A)L23

TOTAL FOR ALL FILES
L36         60 L12(15A) L24

=> s l12 and l24
FILE 'MEDLINE'
L37         25 L1 AND L13

FILE 'SCISEARCH'
L38         17 L2 AND L14

FILE 'LIFESCI'
L39         12 L3 AND L15

FILE 'BIOTECHDS'
L40          5 L4 AND L16

FILE 'BIOSIS'
L41         21 L5 AND L17

FILE 'EMBASE'
L42         19 L6 AND L18

FILE 'HCAPLUS'
L43         46 L7 AND L19

FILE 'NTIS'
L44          0 L8 AND L20

FILE 'ESBIOBASE'
L45         12 L9 AND L21

FILE 'BIOTECHNO'
L46         12 L10 AND L22

FILE 'WPIDS'
L47         13 L11 AND L23
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TOTAL FOR ALL FILES
L48        182 L12 AND L24

=> s hbx
FILE 'MEDLINE'
L49        401 HBX

FILE 'SCISEARCH'
L50        471 HBX

FILE 'LIFESCI'
L51        230 HBX

FILE 'BIOTECHDS'
L52         17 HBX

FILE 'BIOSIS'
L53        412 HBX

FILE 'EMBASE'
L54        352 HBX

FILE 'HCAPLUS'
L55        816 HBX

FILE 'NTIS'
L56         26 HBX

FILE 'ESBIOBASE'
L57        253 HBX

FILE 'BIOTECHNO'
L58        238 HBX

FILE 'WPIDS'
L59         19 HBX

TOTAL FOR ALL FILES
L60       3235 HBX

=> s l60 and l24
FILE 'MEDLINE'
L61         13 L49 AND L13

FILE 'SCISEARCH'
L62         11 L50 AND L14

FILE 'LIFESCI'
L63          8 L51 AND L15

FILE 'BIOTECHDS'
L64          1 L52 AND L16

FILE 'BIOSIS'
L65         11 L53 AND L17

FILE 'EMBASE'
L66         10 L54 AND L18

FILE 'HCAPLUS'
L67         20 L55 AND L19

FILE 'NTIS'
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```
L68           0 L56 AND L20

FILE 'ESBIOBASE'
L69           9 L57 AND L21

FILE 'BIOTECHNO'
L70           7 L58 AND L22

FILE 'WPIDS'
L71           2 L59 AND L23

TOTAL FOR ALL FILES
L72          92 L60 AND L24

=> s l48 or l72
FILE 'MEDLINE'
L73          25 L37 OR L61

FILE 'SCISEARCH'
L74          17 L38 OR L62

FILE 'LIFESCI'
L75          12 L39 OR L63

FILE 'BIOTECHDS'
L76           5 L40 OR L64

FILE 'BIOSIS'
L77          21 L41 OR L65

FILE 'EMBASE'
L78          19 L42 OR L66

FILE 'HCAPLUS'
L79          46 L43 OR L67

FILE 'NTIS'
L80           0 L44 OR L68

FILE 'ESBIOBASE'
L81          12 L45 OR L69

FILE 'BIOTECHNO'
L82          12 L46 OR L70

FILE 'WPIDS'
L83          13 L47 OR L71

TOTAL FOR ALL FILES
L84         182 L48 OR L72

=> dup rem l84
PROCESSING COMPLETED FOR L84
L85          73 DUP REM L84 (109 DUPLICATES REMOVED)

=> d tot

L85  ANSWER 1 OF 73  HCAPLUS  COPYRIGHT 2005 ACS on STN
TI   Method for detection of viruses, food or water contamination and diagnosis
     of diseases using gold particle-labeled antibodies and arrays
SO   PCT Int. Appl., 77 pp.
     CODEN: PIXXD2
IN   Ramael, Marc; Sanders, Jean-paul
AN   2005:182915  HCAPLUS
```

DN 142:276366

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI WO 2005019820 | A1 | 20050303 | WO 2003-EP9393 | 20030825 |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

L85 ANSWER 2 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN
TI Selective pharmacologic inhibition of protein trafficking and related methods of treating human diseases
SO PCT Int. Appl., 134 pp.
CODEN: PIXXD2
IN Sircar, Jagadish; Richards, Mark L.
AN 2005:136530 HCAPLUS
DN 142:233301

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI WO 2005013950 | A2 | 20050217 | WO 2004-US26435 | 20040809 |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

L85 ANSWER 3 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN
TI Prognosis determination in Ewing sarcoma patients by genetic profiling
SO PCT Int. Appl., 58 pp.
CODEN: PIXXD2
IN Avigad, Smadar; Yaniv, Isaac; Zaizov, Rina; Ohali, Anat
AN 2005:34707 HCAPLUS
DN 142:128580

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI WO 2005002414 | A2 | 20050113 | WO 2004-IL578 | 20040630 |
| WO 2005002414 | A3 | 20050310 | | |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

L85 ANSWER 4 OF 73 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
TI New 4,6-disubstituted aminopyrimidine derivatives are protein kinase inhibitors useful for the treatment of e.g. asthma, diabetes, rheumatic

diseases, AIDS, rhinitis and chronic obstructive pulmonary diseases.
PI WO 2005026129 A1 20050324 (200528)* EN 211 C07D239-42
RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NA NL OA PL PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
IN BACKES, A; BRAVO, J; CHOIDAS, A; COTTEN, M; ENGKVIST, O; FELBER, B; FREISLEBEN, A; GODL, K; GREFF, Z; HABENBERGER, P; HAFENBRADL, D; HARRIS, J; HARTUNG, C; HERGET, T; HOPPE, E; KLEBL, B; LE, J; MACRITCHIE, J; MISSIO, A; MUELLER, G; SAVIC, V; SCHWAB, W; SHERBORNE, B; SIMPSON, D; ZECH, B

L85 ANSWER 5 OF 73 MEDLINE on STN DUPLICATE 1
TI Inhibitory effect of cyclosporine A on **hepatitis B virus** replication in vitro and its possible mechanisms.
SO Hepatobiliary & pancreatic diseases international : HBPD INT, (2005 Feb) 4 (1) 18-22. Ref: 47
Journal code: 101151457. ISSN: 1499-3872.
AU Xia Wei-Liang; Shen Yan; Zheng Shu-Sen
AN 2005100531 MEDLINE

L85 ANSWER 6 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 2
TI Gene expression profiles and biomarkers for the detection of hyperlipidemia and other disease-related gene transcripts in blood
SO U.S. Pat. Appl. Publ., 155 pp., Cont.-in-part of U.S. Ser. No. 802,875.
CODEN: USXXCO
IN Liew, Choong-Chin
AN 2005:156681 HCAPLUS
Correction of: 2005:60757
DN 142:216629
Correction of: 142:132329

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI US 2004248170 | A1 | 20041209 | US 2004-812777 | 20040330 |
| US 2004014059 | A1 | 20040122 | US 2002-268730 | 20021009 |
| US 2004248170 | A1 | 20041209 | US 2004-812777 | 20040330 |
| US 2004248170 | A1 | 20041209 | US 2004-812777 | 20040330 |
| US 2004265869 | A1 | 20041230 | US 2004-812716 | 20040330 |

L85 ANSWER 7 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 3
TI Sequences of human schizophrenia related genes and use for diagnosis, prognosis and therapy
SO U.S. Pat. Appl. Publ., 156 pp., Cont.-in-part of U.S. Ser. No. 802,875.
CODEN: USXXCO
IN Liew, Choong-chin
AN 2005:248644 HCAPLUS
DN 142:274057

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI US 2004241727 | A1 | 20041202 | US 2004-812731 | 20040330 |
| US 2004014059 | A1 | 20040122 | US 2002-268730 | 20021009 |
| US 2004241727 | A1 | 20041202 | US 2004-812731 | 20040330 |

L85 ANSWER 8 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN
TI Microarray for determining expression of psychoneuroendocrinimmune genes and diagnosis of diseases
SO PCT Int. Appl., 254 pp.
CODEN: PIXXD2
IN Nicholson, Ainsley; Vernon, Suzanne D.
AN 2004:1081026 HCAPLUS
DN 142:50129

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|---|
| PI | WO 2004108899 | A2 | 20041216 | WO 2004-US17686 | 20040604 |
| | W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW | | | | |
| | RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |

L85 ANSWER 9 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN
TI Differentially regulated nuclear genes encoding mitochondrial proteins in bipolar disorder and their use as markers in diagnosis, monitoring, and therapy
SO PCT Int. Appl., 101 pp.
CODEN: PIXXD2
IN Konradi, Christine; Heckers, Stephan
AN 2004:824003 HCAPLUS
DN 141:312240

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|---|
| PI | WO 2004085614 | A2 | 20041007 | WO 2004-US8516 | 20040319 |
| | W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW | | | | |
| | RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |
| | US 2004248286 | A1 | 20041209 | US 2004-804950 | 20040319 |

L85 ANSWER 10 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN
TI Proteins interacting with the POSH ubiquitin ligase and screening for effectors of the interaction with therapeutic use
SO PCT Int. Appl., 374 pp.
CODEN: PIXXD2
IN Taglicht, Daniel N.; Alroy, Iris; Reiss, Yuval; Yaar, Liora; Ben-Avraham, Danny; Tuvia, Shmuel; Greener, Tsvika
AN 2004:756607 HCAPLUS
DN 141:273624

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|---|
| PI | WO 2004078130 | A2 | 20040916 | WO 2004-US6308 | 20040302 |
| | W: AE, AE, AG, AL, AL, AM, AM, AM, AT, AT, AU, AZ, AZ, BA, BB, BG, BG, BR, BR, BW, BY, BY, BZ, BZ, CA, CH, CN, CN, CO, CO, CR, CR, CU, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EC, EE, EE, EG, ES, ES, FI, FI, GB, GD, GE, GE, GH, GM, HR, HR, HU, HU, ID, IL, IN, IS, JP, JP, KE, KE, KG, KG, KP, KP, KP, KR, KR, KZ, KZ, KZ, LC, LK, LR, LS, LS, LT, LU, LV, MA, MD, MD, MG, MK, MN, MW, MX, MX, MZ, MZ, NA, NI | | | | |
| | RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |

WO 2004098492 A2 20041118 WO 2003-US335712 20031110
WO 2004098492 A3 20050428
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
WO 2004073609 A2 20040902 WO 2004-US3600 20040205
W: AE, AE, AG, AL, AL, AM, AM, AM, AT, AT, AU, AZ, AZ, BA, BB, BG, BG, BR, BR, BW, BY, BY, BZ, BZ, CA, CH, CN, CN, CO, CO, CR, CR, CU, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EC, EE, EE, EG, ES, ES, FI, FI, GB, GD, GE, GE, GH, GM, HR, HR, HU, HU, ID, IL, IN, IS, JP, JP, KE, KE, KG, KG, KP, KP, KP, KR, KR, KZ, KZ, KZ, LC, LK, LR, LS, LS, LT, LU, LV, MA, MD, MD, MG, MK, MN, MW, MX, MX, MZ, MZ, NA, NI
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
WO 2004089302 A2 20041021 WO 2004-US10582 20040405
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
WO 2005007141 A2 20050127 WO 2004-US21900 20040709
WO 2005007141 A3 20050324
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

L85 ANSWER 11 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN
TI compns. comprising polyphenolic compds. and inhibitors of ROS for diseases involving NF-κB activation
SO U.S. Pat. Appl. Publ., 80 pp., Cont.-in-part of U.S. Ser. No. 260,609.
CODEN: USXXCO
IN Pandol, Stephen J.; Gukovskaya, Anna; Yazbeck, Moussa; Eibl, Guido; Boros, Laszlo G.
AN 2004:1127079 HCAPLUS
DN 142:69216

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|---|
| PI | US 2004259816 | A1 | 20041223 | US 2004-824597 | 20040415 |
| | US 2004063648 | A1 | 20040401 | US 2002-260609 | 20021001 |

L85 ANSWER 12 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN
TI Identification and mapping of peptide epitopes
SO U.S., 16 pp., Cont.-in-part of U.S. 6,070,126.
CODEN: USXXAM
IN Kokolus, William J.
AN 2004:691451 HCAPLUS
DN 141:189618

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| US 6780598 | B1 | 20040824 | US 2000-552461 | 20000418 |
| US 6070126 | A | 20000530 | US 1998-97078 | 19980612 |
| CA 2370760 | AA | 20001026 | CA 2000-2370760 | 20000419 |
| WO 2000063693 | A1 | 20001026 | WO 2000-US10585 | 20000419 |

PI
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

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|---|---|---|---|---|
| EP 1179178 | A1 | 20020213 | EP 2000-928224 | 20000419 |

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

L85 ANSWER 13 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN
TI Pharmaceutical composition of interferon gamma with molecular diagnostics for the improved treatment of bronchial asthma
SO Eur. Pat. Appl., 23 pp.
CODEN: EPXXDW
IN Bevec, Dorian; Ziesche, Rolf
AN 2004:510134 HCAPLUS
DN 141:52871

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| EP 1430902 | A1 | 20040623 | EP 2002-28574 | 20021220 |

PI
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK

L85 ANSWER 14 OF 73 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
TI New (1,2,4)thiadiazinyl-1H-pyridin-2-one derivatives useful as anti-infective agents in the treatment of e.g. hepatitis C virus infection.
PI US 2004087577 A1 20040506 (200436)* 160 A61K031-549
IN BETEBENNER, D A; DONNER, P L; GREEN, B E; KEMPF, D J; MARING, C J; MCDANIEL, K F; PRATT, J K; STOLL, V S; ZHANG, R

L85 ANSWER 15 OF 73 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
TI Molecular mechanism for the potentiation of the transcriptional activity of human liver receptor homolog 1 by steroid receptor coactivator-1
SO MOLECULAR ENDOCRINOLOGY, (AUG 2004) Vol. 18, No. 8, pp. 1887-1905.
Publisher: ENDOCRINE SOC, 8401 CONNECTICUT AVE, SUITE 900, CHEVY CHASE, MD 20815-5817 USA.
ISSN: 0888-8809.
AU Xu P L; Liu Y Q; Shan S F; Kong Y Y; Zhou Q; Li M; Ding J P (Reprint); Xie Y H; Wang Y
AN 2004:705822 SCISEARCH

L85 ANSWER 16 OF 73 MEDLINE on STN DUPLICATE 4
TI **Hepatitis B virus** X protein is essential for the activation of Wnt/beta-catenin signaling in hepatoma cells.

SO Hepatology (Baltimore, Md.), (2004 Jun) 39 (6) 1683-93.
Journal code: 8302946. ISSN: 0270-9139.
AU Cha Man-Young; Kim Chang-Myeong; Park Young-Min; Ryu Wang-Shick
AN 2004283685 MEDLINE

L85 ANSWER 17 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN
TI Methods for production and use of mammalian complementarity determining region mimetibodies for diagnosis and therapy of human diseases
SO PCT Int. Appl., 97 pp.
CODEN: PIXXD2
IN Heavner, George A.; Knight, David M.; Scallon, Bernard J.; Ghrayeb, John
AN 2003:818235 HCAPLUS
DN 139:322283

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI WO 2003084477 | A2 | 20031016 | WO 2003-US9139 | 20030324 |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

L85 ANSWER 18 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN
TI Isolation of ligands capable of binding to MHC/HLA molecule for use as HLA-restricted vaccines
SO PCT Int. Appl., 98 pp.
CODEN: PIXXD2
IN Klade, Christoph; Schalich, Juliane; Vytvytska, Oresta; Zauner, Wolfgang; Birnstiel, Max; Aichinger, Gerald; Otava, Alexander; Mattner, Frank
AN 2003:697157 HCAPLUS
DN 139:229251

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI WO 2003073097 | A2 | 20030904 | WO 2003-EP2005 | 20030227 |
| WO 2003073097 | C2 | 20031224 | | |
| WO 2003073097 | A3 | 20040930 | | |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

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|---|---|---|---|---|
| CA 2476571 | AA | 20030904 | CA 2003-2476571 | 20030227 |
| EP 1483575 | A2 | 20041208 | EP 2003-709724 | 20030227 |

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK

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|---|---|---|---|---|
| CA 2484339 | AA | 20040325 | CA 2003-2484339 | 20030827 |
| WO 2004024182 | A2 | 20040325 | WO 2003-EP9482 | 20030827 |
| WO 2004024182 | A3 | 20041223 | | |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

EP 1537418 A2 20050608 EP 2003-794948 20030827

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK

L85 ANSWER 19 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN

TI Novel pharmaceutical composition of interferon gamma or pirfenidone combined with molecular diagnostics for the improved treatment of interstitial lung diseases

SO PCT Int. Appl., 80 pp.
CODEN: PIXXD2

IN Bevec, Dorian; Ziesche, Rolf

AN 2003:491063 HCAPLUS

DN 139:57897

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2003051388 | A2 | 20030626 | WO 2002-CH691 | 20021212 |
| WO 2003051388 | A3 | 20031030 | | |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

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|---|---|---|---|---|
| CA 2470763 | AA | 20030626 | CA 2002-2470763 | 20021212 |
| BR 2002007310 | A | 20040817 | BR 2002-7310 | 20021212 |
| EP 1455813 | A2 | 20040915 | EP 2002-782602 | 20021212 |

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK

NO 2003003642 A 20031017 NO 2003-3642 20030815

L85 ANSWER 20 OF 73 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN

TI Use of mappicine analogs for treatment of a patient infected with a retrovirus or **hepadnavirus** e.g. HIV and human **hepatitis B virus.**

PI WO 2003103610 A2 20031218 (200408)* EN 56 A61K000-00

RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG UZ VC VN YU ZA ZM ZW

US 2004058948 A1 20040325 (200422) A61K031-4745

AU 2003265223 A1 20031222 (200445) A61K000-00

IN CURRAN, D P; GABARDA, A; PARNIAK, M A

L85 ANSWER 21 OF 73 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN

TI Nucleoside library useful for the treatment of viral infections and neoplastic diseases comprises two library compounds containing a sugar that is covalently bound to a purine having a substituent in the second position.

PI WO 2003051881 A1 20030626 (200356)* EN 57 C07D473-00

RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SI SK SL SZ TR TZ UG ZM ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR

KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA
ZM ZW

AU 2002359732 A1 20030630 (200420) C07D473-00

IN AN, H; BARAWKAR, D; CHEN, H; GIRARDET, J; GUNIC, E; HONG, Z; KOH, Y; RONG, F; ZHANG, W

L85 ANSWER 22 OF 73 MEDLINE on STN DUPLICATE 5

TI **Hepatitis B virus** X protein activates a survival signaling by linking **SRC** to phosphatidylinositol 3-kinase.

SO Journal of biological chemistry, (2003 Aug 22) 278 (34) 31807-13. Electronic Publication: 2003-06-12. Journal code: 2985121R. ISSN: 0021-9258.

AU Shih Wen-Ling; Kuo Min-Liang; Chuang Shuang-En; Cheng Ann-Lii; Doong Shin-Lian

AN 2003401413 MEDLINE

L85 ANSWER 23 OF 73 MEDLINE on STN DUPLICATE 6

TI Activation and inhibition of cellular calcium and tyrosine kinase signaling pathways identify targets of the **HBx** protein involved in **hepatitis B virus** replication.

SO Journal of virology, (2003 Jul) 77 (14) 7713-9. Journal code: 0113724. ISSN: 0022-538X.

AU Bouchard Michael J; Puro Robyn J; Wang Lihua; Schneider Robert J

AN 2003301915 MEDLINE

L85 ANSWER 24 OF 73 MEDLINE on STN DUPLICATE 7

TI Characterization of a strong repression domain in the hinge region of orphan nuclear receptor hB1F/hLRH-1.

SO Sheng wu hua xue yu sheng wu wu li xue bao Acta biochimica et biophysica Sinica, (2003 Oct) 35 (10) 909-16. Journal code: 20730160R. ISSN: 0582-9879.

AU Xu Ping-Long; Shan Shi-Fang; Kong Yu-Ying; Xie You-Hua; Wang Yuan

AN 2003454650 MEDLINE

L85 ANSWER 25 OF 73 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

TI New polynucleotides, useful to detect extrachromosomal molecules and screening for modulating agents e.g. anticancer agents, comprises extrachromosomal molecule operably linked to tag;
recombinant polynucleotide production, vector expression in host cell, fluorescent protein useful in disease gene therapy and drug screening

AU KANDA T; WAHL G M; FASEL-OTTER M

AN 2002-12409 BIOTECHDS

PI WO 2002020823 14 Mar 2002

L85 ANSWER 26 OF 73 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

TI Treating **Hepatitis B virus** infection and hepatocellular carcinoma, by administering a compound that modulates the synthesis or expression of a target cellular gene or the activity of a target protein, e.g. Pyk2 kinase;
enzyme gene expression modulation and inhibition and sense and antisense sequence use in disease therapy and gene therapy

AU SCHNEIDER R J; KLEIN N

AN 2002-16437 BIOTECHDS

PI US 2002045191 18 Apr 2002

L85 ANSWER 27 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 10

TI Preparation of benzo[g]quinoxalines for use against infectious diseases

SO PCT Int. Appl., 237 pp. CODEN: PIXXD2

IN Pato, Janos; Keri, Gyoergy; Oerfi, Laszlo; Waczek, Frigyes; Horvath, Zoltan; Banhegyi, Peter; Szabadkai, Istvan; Marosfalvi, Jenoe; Hegymegi-barakonyi, Balint; Szekelyhidi, Zsolt; Greff, Zoltan; Choidas,

Axel; Bacher, Gerald; Daub, Henrik; Obert, Sabine; Kurtenbach, Alexander; Habenberger, Peter

AN 2002:906175 HCAPLUS

DN 138:14074

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI WO 2002094796 | A2 | 20021128 | WO 2002-EP5573 | 20020521 |
| WO 2002094796 | A3 | 20031204 | | |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

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|---|---|---|---|---|
| US 2004171603 | A1 | 20040902 | US 2003-715591 | 20031118 |

L85 ANSWER 28 OF 73 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

TI Detecting target nucleic acid during amplification, useful e.g. for detecting mutations, uses double strand-specific, heat-labile fluorescent dye;
DNA primer and polymerase chain reaction for target DNA and mutation detection

AU WITTWER C T; RIRIE K M; RASMUSSEN R P

AN 2002-18131 BIOTECHDS

PI US 2002058258 16 May 2002

L85 ANSWER 29 OF 73 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

TI Inhibition of the **Src** kinase family pathway as a method of treating **HBV** infection and hepatocellular carcinoma.

SO Official Gazette of the United States Patent and Trademark Office Patents, (July 16, 2002) Vol. 1260, No. 3. http://www.uspto.gov/web/menu/patdata.html. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.

AU Schneider, Robert J. [Inventor, Reprint author]; Klein, Nicola [Inventor]

AN 2002:475362 BIOSIS

L85 ANSWER 30 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN

TI Drug design against drug resistant mutants using directed evolution and target protein conformation changes

SO PCT Int. Appl., 82 pp.
CODEN: PIXXD2

IN Stevens, Raymond C.; Orencia, Maria C.; Yoon, Jun S.; Hanson, Michael A.

AN 2002:676289 HCAPLUS

DN 137:211942

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI WO 2002068933 | A2 | 20020906 | WO 2002-US6238 | 20020227 |
| WO 2002068933 | A3 | 20021121 | | |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

L85 ANSWER 31 OF 73 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
TI New thienopyrimidine-based analog compounds useful for treating e.g. hyperproliferative disease, hematologic diseases, osteoporosis, neurological disease, autoimmune disease, bacterial infection or viral infections.
PI WO 2002057271 A2 20020725 (200267)* EN 155 C07D495-04
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PH PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
US 6503914 B1 20030107 (200306) C07D495-04
KR 2003040554 A 20030522 (200360) C07D495-04
AU 2002246817 A1 20020730 (200427) C07D495-04
EP 1409491 A2 20040421 (200427) EN C07D495-04
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR
US 2004077663 A1 20040422 (200428) A61K031-519
JP 2004517898 W 20040617 (200440) 285 C07D495-04
IN BENISH, M A; BUDDE, R J A; LAWLESS, M; BUDDE, R J

L85 ANSWER 32 OF 73 MEDLINE on STN DUPLICATE 11
TI **Hepatitis B virus** X protein activates the p38 mitogen-activated protein kinase pathway in dedifferentiated hepatocytes.
SO Journal of virology, (2002 Oct) 76 (19) 9763-72.
Journal code: 0113724. ISSN: 0022-538X.
AU Tarn Chi; Zou Lin; Hullinger Ronald L; Andrisani Ourania M
AN 2002454084 MEDLINE

L85 ANSWER 33 OF 73 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 12
TI Chronic viral hepatitis: Liver biopsy, **yes** or no?.
SO Revista Espanola de Enfermedades Digestivas, (Octubre 2002) Vol. 94, No. 10, pp. 619-624. print.
CODEN: REDIEM. ISSN: 1130-0108.
AU Castellano Tortajada, G. [Reprint Author]
AN 2003:157049 BIOSIS

L85 ANSWER 34 OF 73 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
TI A dipalmitoyl peptide that binds SH3 domain, disturbs intracellular signal transduction, and inhibits tumor growth in vivo
SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (16 AUG 2002) Vol. 296, No. 2, pp. 434-442.
Publisher: ACADEMIC PRESS INC ELSEVIER SCIENCE, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA.
ISSN: 0006-291X.
AU Lee K Y; Yoon J H; Kim M; Roh S; Lee Y S; Seong B L; Kim K (Reprint)
AN 2002:703240 SCISEARCH

L85 ANSWER 35 OF 73 MEDLINE on STN
TI Gene expression profile in response to **hepatitis B virus** X gene by using an adenoviral vector.
SO Taehan Kan Hakhoe chi = Korean journal of hepatology, (2002 Dec) 8 (4) 371-80.
Journal code: 9607534. ISSN: 1226-0479.
AU Joo Heui Yun; Han Kwang Hyub; Ryu Wang Shick
AN 2002742604 MEDLINE

L85 ANSWER 36 OF 73 MEDLINE on STN
TI Recombinant hepatitis B triple antigen vaccine: Hepacare.
SO Expert review of vaccines, (2002 Aug) 1 (2) 141-4. Ref: 20
Journal code: 101155475. ISSN: 1476-0584.

AU Zuckerman Jane N; Zuckerman Arie J
AN 2003367292 MEDLINE

L85 ANSWER 37 OF 73 MEDLINE on STN
TI Virology. The X files--one step closer to closure.
SO Science, (2001 Dec 14) 294 (5550) 2299-300.
Journal code: 0404511. ISSN: 0036-8075.
AU Ganem D
AN 2001695469 MEDLINE

L85 ANSWER 38 OF 73 MEDLINE on STN DUPLICATE 13
TI Identification of a conserved residue of foamy virus Gag required for intracellular capsid assembly.
SO Journal of virology, (2001 Aug) 75 (15) 6857-64.
Journal code: 0113724. ISSN: 0022-538X.
AU Eastman S W; Linial M L
AN 2001379350 MEDLINE

L85 ANSWER 39 OF 73 MEDLINE on STN DUPLICATE 14
TI **Hepatitis B virus HBx** protein
activation of cyclin A-cyclin-dependent kinase 2 complexes and G1 transit via a **Src** kinase pathway.
SO Journal of virology, (2001 May) 75 (9) 4247-57.
Journal code: 0113724. ISSN: 0022-538X.
AU Bouchard M; Giannakopoulos S; Wang E H; Tanese N; Schneider R J
AN 2001200595 MEDLINE

L85 ANSWER 40 OF 73 MEDLINE on STN DUPLICATE 15
TI The **hepatitis B virus HBx** protein
induces adherens junction disruption in a **src**-dependent manner.
SO Oncogene, (2001 Jun 7) 20 (26) 3323-31.
Journal code: 8711562. ISSN: 0950-9232.
AU Lara-Pezzi E; Roche S; Andrisani O M; Sanchez-Madrid F; Lopez-Cabrera M
AN 2001360694 MEDLINE

L85 ANSWER 41 OF 73 MEDLINE on STN DUPLICATE 16
TI Calcium signaling by **HBx** protein in **hepatitis B virus** DNA replication.
SO Science, (2001 Dec 14) 294 (5550) 2376-8.
Journal code: 0404511. ISSN: 0036-8075.
AU Bouchard M J; Wang L H; Schneider R J
AN 2001695488 MEDLINE

L85 ANSWER 42 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN
TI Virology: The X-files - one step closer to closure
SO Science (Washington, DC, United States) (2001), 294(5550), 2299-2300
CODEN: SCIEAS; ISSN: 0036-8075
AU Ganem, Don
AN 2001:915723 HCAPLUS
DN 136:181399

L85 ANSWER 43 OF 73 MEDLINE on STN
TI Through induction of juxtaposition and tyrosine kinase activity of Jak1, X-gene product of **hepatitis B virus**
stimulates Ras and the transcriptional activation through AP-1, NF-kappaB, and SRE enhancers.
SO Biochemical and biophysical research communications, (2001 Sep 7) 286 (5) 886-94.
Journal code: 0372516. ISSN: 0006-291X.
AU Kim H; Lee Y H; Won J; Yun Y
AN 2001485447 MEDLINE

L85 ANSWER 44 OF 73 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

TI Foamy virus assembly.
SO Virus Research, (October, 2001) Vol. 77, No. 2, pp. 118-119. print.
Meeting Info.: Retrovirus Assembly Meeting. Prague, Czech Republic. October 14-18, 2000.
CODEN: VIREDF. ISSN: 0168-1702.
AU Eastman, S. [Reprint author]; Linial, M. L. [Reprint author]
AN 2001:559059 BIOSIS

L85 ANSWER 45 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 17
TI Compounds and methods for genetic immunization
SO PCT Int. Appl., 41 pp.
CODEN: PIXXD2
IN Gebhard, John; Araneo, Barbara A.
AN 2000:314577 HCAPLUS
DN 132:333379

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI WO 2000025820 | A1 | 20000511 | WO 1999-US25979 | 19991103 |

W: AU, CA, JP
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

L85 ANSWER 46 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN
TI Modified peptides containing an antibody Fc domain as therapeutic agents
SO PCT Int. Appl., 608 pp.
CODEN: PIXXD2
IN Feige, Ulrich; Liu, Chuan-fa; Cheetham, Janet; Boone, Thomas Charles
AN 2000:291095 HCAPLUS
DN 132:329919

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI WO 2000024782 | A2 | 20000504 | WO 1999-US25044 | 19991025 |
| WO 2000024782 | A3 | 20020606 | | |

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

| | | | | |
|---|---|---|---|---|
| US 6660843 | B1 | 20031209 | US 1999-428082 | 19991022 |
| CA 2347131 | AA | 20000504 | CA 1999-2347131 | 19991025 |
| EP 1144454 | A2 | 20011017 | EP 1999-971003 | 19991025 |
| EP 1144454 | A3 | 20020911 | | |

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

| | | | | |
|---|---|---|---|---|
| BR 9914708 | A | 20020716 | BR 1999-14708 | 19991025 |
| JP 2003512011 | T2 | 20030402 | JP 2000-578351 | 19991025 |
| AU 767725 | B2 | 20031120 | AU 2000-12322 | 19991025 |
| NZ 510888 | A | 20040130 | NZ 1999-510888 | 19991025 |
| ZA 2001002753 | A | 20020611 | ZA 2001-2753 | 20010404 |
| NO 2001001963 | A | 20010621 | NO 2001-1963 | 20010420 |
| BG 105461 | A | 20030430 | BG 2001-105461 | 20010424 |
| US 2004044188 | A1 | 20040304 | US 2003-609217 | 20030627 |
| US 2004053845 | A1 | 20040318 | US 2003-632388 | 20030731 |
| US 2004071712 | A1 | 20040415 | US 2003-645761 | 20030818 |
| US 2005123548 | A1 | 20050609 | US 2003-645784 | 20030818 |
| US 2004057953 | A1 | 20040325 | US 2003-651723 | 20030829 |
| US 2004087778 | A1 | 20040506 | US 2003-653048 | 20030829 |
| US 2004077022 | A1 | 20040422 | US 2003-666696 | 20030919 |

L85 ANSWER 47 OF 73 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN

TI Treating hyperproliferative or pathogenic diseases involves administration of an expression construct comprising a self-gene or pathogen gene under the control of a promoter.

PI WO 2000054839 A2 20000921 (200063)* EN 104 A61K048-00
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2000037558 A 20001004 (200101) A61K048-00
EP 1165144 A2 20020102 (200209) EN A61K048-00
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
US 2003045499 A1 20030306 (200320) A61K048-00

IN CARBONE, D; CHADA, S; GABRILOVICH, D; MHASHILKAR, A

L85 ANSWER 48 OF 73 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN

TI Determining the length of amino acid residues and identifying immunobiologically-active linear peptide epitopes of a protein antigen comprises applying a custom negative cosine fit algorithm to a protein hydropathy scale.

PI US 6070126 A 20000530 (200035)* 19 C07K014-00

IN FRITSCHE, H A; JOHNSTON, D A; KOKOLUS, W J

L85 ANSWER 49 OF 73 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

TI Putative role of **hepatitis B virus** X protein in hepatocarcinogenesis: Effects on apoptosis, DNA repair, mitogen-activated protein kinase and JAK/STAT pathways

SO JOURNAL OF GASTROENTEROLOGY AND HEPATOLOGY, (APR 2000) Vol. 15, No. 4, pp. 357-368.
Publisher: BLACKWELL SCIENCE ASIA, 54 UNIVERSITY ST, P O BOX 378, CARLTON VICTORIA 3053, AUSTRALIA.
ISSN: 0815-9319.

AU Arbuthnot P; Capovilla A; Kew M (Reprint)

AN 2000:283755 SCISEARCH

L85 ANSWER 50 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN

TI Oncogene or virus induced multistep expression systems for gene therapy

SO Eur. Pat. Appl., 44 pp.
CODEN: EPXXDW

IN Muller, Rolf; Sedlacek, Hans-Harald

AN 1999:393034 HCAPLUS

DN 131:40554

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI EP 922768 | A2 | 19990616 | EP 1998-121471 | 19981111 |
| EP 922768 | A3 | 20000105 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO | | | | |
| DE 19751587 | A1 | 19990729 | DE 1997-19751587 | 19971121 |
| CA 2251257 | AA | 19990521 | CA 1998-2251257 | 19981119 |
| AU 9893256 | A1 | 19990610 | AU 1998-93256 | 19981119 |
| AU 745614 | B2 | 20020328 | | |
| CN 1221033 | A | 19990630 | CN 1998-122537 | 19981120 |
| BR 9804720 | A | 20000328 | BR 1998-4720 | 19981120 |
| US 6465246 | B1 | 20021015 | US 1998-196099 | 19981120 |
| JP 2000106886 | A2 | 20000418 | JP 1998-333200 | 19981124 |

L85 ANSWER 51 OF 73 MEDLINE on STN DUPLICATE 18

TI **Src** kinases involved in **hepatitis B virus** replication.

SO EMBO journal, (1999 Sep 15) 18 (18) 5019-27.
Journal code: 8208664. ISSN: 0261-4189.

AU Klein N P; Bouchard M J; Wang L H; Kobarg C; Schneider R J
AN 1999417583 MEDLINE

L85 ANSWER 52 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 19
TI Inhibition of the **Src** kinase family pathway as a method of treating **hepatitis B virus** infection and hepatocellular carcinoma
SO PCT Int. Appl., 85 pp.
CODEN: PIXXD2
IN Schneider, Robert J.; Klein, Nicola
AN 1999:8208 HCAPLUS
DN 130:61060

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI WO 9857175 | A1 | 19981217 | WO 1998-US12279 | 19980612 |
| W: AU, CA, JP | | | | |
| RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| US 6420338 | B1 | 20020716 | US 1997-874430 | 19970613 |
| CA 2293350 | AA | 19981217 | CA 1998-2293350 | 19980612 |
| AU 9878385 | A1 | 19981230 | AU 1998-78385 | 19980612 |
| AU 757164 | B2 | 20030206 | | |
| EP 988548 | A1 | 20000329 | EP 1998-926584 | 19980612 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI | | | | |
| US 2003032596 | A1 | 20030213 | US 2002-196344 | 20020715 |

L85 ANSWER 53 OF 73 MEDLINE on STN DUPLICATE 20
TI X-gene product of **hepatitis B virus** induces apoptosis in liver cells.
SO Journal of biological chemistry, (1998 Jan 2) 273 (1) 381-5.
Journal code: 2985121R. ISSN: 0021-9258.
AU Kim H; Lee H; Yun Y
AN 1998079072 MEDLINE

L85 ANSWER 54 OF 73 MEDLINE on STN
TI Preventive measures against **hepatitis B virus** infection in nursing schools in Japan.
SO [Nippon koshu eisei zasshi] Japanese journal of public health, (1998 Jan) 45 (1) 67-72.
Journal code: 19130150R. ISSN: 0546-1766.
AU Morishita M; Kamachi C; Imamura T; Matsuu K
AN 1998214011 MEDLINE

L85 ANSWER 55 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN
TI Activation of c-**src** by the **hepatitis B virus** (**HBx**) protein is essential for ras activation, cell cycle progression and viral replication
SO (1997) 219 pp. Avail.: UMI, Order No. DA9731409
From: Diss. Abstr. Int., B 1997, 58(4), 1696
AU Klein, Nicola Penny
AN 1997:662018 HCAPLUS
DN 127:258578

L85 ANSWER 56 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN
TI Prophylactic and therapeutic vector vaccination using expression constructs for individual epitopes of antigens
SO U.S., 50 pp., Cont.-in-part of U.S. Ser. No. 29,336, abandoned.
CODEN: USXXAM
IN Weiner, David B.; Williams, William V.; Wang, Bin
AN 1997:97727 HCAPLUS
DN 126:156420

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|

PI US 5593972 A 19970114 US 1993-125012 19930921
ZA 9400493 A 19950103 ZA 1994-493 19940125
CA 2153593 AA 19940804 CA 1994-2153593 19940126
WO 9416737 A1 19940804 WO 1994-US899 19940126
W: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, US, US, US, US, UZ, VN
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG
AU 9462320 A1 19940815 AU 1994-62320 19940126
AU 675702 B2 19970213
EP 681483 A1 19951115 EP 1994-909492 19940126
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE
HU 73099 A2 19960628 HU 1995-2229 19940126
HU 219767 B 20010730
JP 08509694 T2 19961015 JP 1994-517285 19940126
EP 1473369 A2 20041103 EP 2004-75092 19940126
EP 1473369 A3 20050302
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE
US 6348449 B1 20020219 US 1994-357398 19941216
US 5830876 A 19981103 US 1995-453349 19950530
US 5817637 A 19981006 US 1997-783818 19970113
US 6468982 B1 20021022 US 1997-880576 19970623
US 5981505 A 19991109 US 1997-979385 19971126

L85 ANSWER 57 OF 73 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
TI Preparation of polymerised vaccines which induce TH1 immune response - comprises polymerising peptide(s) or proteins of specified mol.weight , by gradual synthesis or poly-condensn., using polymerising agents in restrictive concentration.
PI WO 9702838 A1 19970130 (199712)* ES 46 A61K039-385
RW: AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE
W: CA CN JP RU US
EP 782860 A1 19970709 (199732) EN 23 A61K039-385
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
ES 2108614 A1 19971216 (199806) A61K039-385
ES 2108614 B1 19980716 (199835) A61K039-385
IN MARTIN, ONCINA F J

L85 ANSWER 58 OF 73 MEDLINE on STN DUPLICATE 21
TI Activation of **Src** family kinases by **hepatitis B virus HBx** protein and coupled signaling to Ras.
SO Molecular and cellular biology, (1997 Nov) 17 (11) 6427-36.
Journal code: 8109087. ISSN: 0270-7306.
AU Klein N P; Schneider R J
AN 1998001570 MEDLINE

L85 ANSWER 59 OF 73 MEDLINE on STN DUPLICATE 22
TI Increased enzymatic activity of the T-cell antigen receptor-associated **fyn** protein tyrosine kinase in asymptomatic patients infected with the human immunodeficiency virus.
SO Blood, (1997 Nov 1) 90 (9) 3603-12.
Journal code: 7603509. ISSN: 0006-4971.
AU Phipps D J; Yousefi S; Branch D R
AN 1998008125 MEDLINE

L85 ANSWER 60 OF 73 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 23
TI Could thymostimulin prevent hepatocellular carcinoma occurrence in patients with liver cirrhosis?.
SO Oncology Reports, (1996) Vol. 3, No. 4, pp. 655-656.
ISSN: 1021-335X.
AU Palmieri, Giovannella [Reprint author]; Biondi, Edoardo; Morrabito,

Alessandro; Rea, Antonio; Bianco, A. Raffaele
AN 1996:368405 BIOSIS

L85 ANSWER 61 OF 73 MEDLINE on STN
TI Should liver transplantation be performed for patients with chronic hepatitis B? **Yes**!.
SO Liver transplantation and surgery : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society, (1995 Jul) 1 (4) 260-5. Ref: 26
Journal code: 9502504. ISSN: 1074-3022.
AU Lake J R
AN 1998003183 MEDLINE

L85 ANSWER 62 OF 73 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
TI Method for introducing genetic material into cell; transfection for genetic immunization using e.g. HIV virus or autoimmune disease antigen DNA administered with bupivacaine
AN 1994-12274 BIOTECHDS
PI WO 9416737 4 Aug 1994

L85 ANSWER 63 OF 73 MEDLINE on STN DUPLICATE 24
TI Minor envelope proteins of duck **hepatitis B virus** are initiated at internal pre-S AUG codons but are not essential for infectivity.
SO Virology, (1993 Nov) 197 (1) 64-73.
Journal code: 0110674. ISSN: 0042-6822.
AU Fernholz D; Wildner G; Will H
AN 94025612 MEDLINE

L85 ANSWER 64 OF 73 MEDLINE on STN
TI Multiple oncogenes and tumor suppressor genes are structurally and functionally intact during hepatocarcinogenesis in **hepatitis B virus** transgenic mice.
SO Cancer research, (1992 May 15) 52 (10) 2823-9.
Journal code: 2984705R. ISSN: 0008-5472.
AU Pasquinelli C; Bhavani K; Chisari F V
AN 92257459 MEDLINE

L85 ANSWER 65 OF 73 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 25
TI EFFECT OF CYTOKINES AND OTHER FACTORS ON THE PRES1 AND PRES2 PROMOTER ACTIVITIES OF THE **HEPATITIS B VIRUS** SUBTYPE ADR.
SO Journal of Catholic Medical College, (1992) Vol. 45, No. 2, pp. 491-499, 501-502.
CODEN: KTUNAA. ISSN: 0368-7015.
AU LEE Y S [Reprint author]
AN 1992:500330 BIOSIS

L85 ANSWER 66 OF 73 LIFESCI COPYRIGHT 2005 CSA on STN
TI Expression of oncogenes and tumor suppressor genes in human hepatocellular carcinoma and hepatoblastoma cell lines.
SO J. MED. VIROL., (1992) vol. 38, no. 4, pp. 325-339.
AU Farshid, M.; Tabor, E.
AN 93:107063 LIFESCI

L85 ANSWER 67 OF 73 MEDLINE on STN DUPLICATE 26
TI Expression of oncogenes and tumor suppressor genes in human hepatocellular carcinoma and hepatoblastoma cell lines.
SO Journal of medical virology, (1992 Dec) 38 (4) 235-9.
Journal code: 7705876. ISSN: 0146-6615.
AU Farshid M; Tabor E
AN 93115701 MEDLINE

L85 ANSWER 68 OF 73 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
TI Nutrient media for cultivation of the recombinant strain Saccharomyces cerevisiae - producer of antigen of **hepatitis B virus**;
culture medium optimization for recombinant vaccine production
SO Biotekhnologiya; (1992) 6, 73-75
CODEN: BTKNEZ
AU Mikhailova L A; Borisova V N
AN 1993-06276 BIOTECHDS

L85 ANSWER 69 OF 73 MEDLINE on STN DUPLICATE 27
TI Absence of genomes of DNA tumor viruses and expression of oncogenes and growth factors in two esophageal carcinoma cell lines of Chinese origin.
SO Zhonghua min guo wei sheng wu ji mian yi xue za zhi = Chinese journal of microbiology and immunology, (1992 May) 25 (2) 59-68.
Journal code: 8008067. ISSN: 0253-2662.
AU Wong F H; Hu C P; Chen S C; Yu Y T; Chang C
AN 93114126 MEDLINE

L85 ANSWER 70 OF 73 HCAPLUS COPYRIGHT 2005 ACS on STN
TI Method and kit for nucleic acid hybridization assay using solubilization with chaotropic salts
SO PCT Int. Appl., 128 pp.
CODEN: PIXXD2
IN Gillespie, David
AN 1988:91391 HCAPLUS
DN 108:91391

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI WO 8706621 | A1 | 19871105 | WO 1987-US1023 | 19870504 |
| W: AU, JP, US | | | | |
| RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE | | | | |
| CA 1301606 | A1 | 19920526 | CA 1987-536166 | 19870501 |
| AU 8774329 | A1 | 19871124 | AU 1987-74329 | 19870504 |
| AU 613870 | B2 | 19910815 | | |
| EP 305399 | A1 | 19890308 | EP 1987-903558 | 19870504 |
| EP 305399 | B1 | 19941123 | | |
| R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE | | | | |
| JP 01502317 | T2 | 19890817 | JP 1987-503095 | 19870504 |
| JP 2552691 | B2 | 19961113 | | |

L85 ANSWER 71 OF 73 MEDLINE on STN
TI A human nucleolar antigen (pp90) associated with cell growth and its induction by Epstein-Barr virus and human cytomegalovirus.
SO International journal of cancer. Journal international du cancer, (1984 Nov 15) 34 (5) 657-65.
Journal code: 0042124. ISSN: 0020-7136.
AU Kamata T; Ohtsuka M; Watanabe Y
AN 85053572 MEDLINE

L85 ANSWER 72 OF 73 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI [Immunizations: **Yes** or no?].
IMPFUNGEN - JA ODER NEIN?.
SO Therapiewoche, (1983) Vol. 33, No. 11, pp. 1401-1408.
CODEN: THEWA6
AU Stickl H.
AN 83099816 EMBASE

L85 ANSWER 73 OF 73 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI [Drug treatment of chronic-active virus hepatitis: **yes** or no?].
SOLL DIE CHRONISCH-AKTIVE VIRUS-HEPATITIS MEDIKAMENTOS BEHANDELT WERDEN ODER NICHT?.

SO Internist, (1981) Vol. 22, No. 12, pp. 717-720.
CODEN: INTEAG
AU Schalm S.W.
AN 82121660 EMBASE

=>
=> d ab 14,20,22,23,32,35,39-41,51,53,58,64

L85 ANSWER 14 OF 73 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
AB US2004087577 A UPAB: 20040611
NOVELTY - (1,2,4)Thiadiazinyl-1H-pyridin-2-one derivatives and their salts, stereoisomers and tautomers are new.

DETAILED DESCRIPTION - (1,2,4)Thiadiazinyl-1H-pyridin-2-one derivatives of formula (I) and their salts, stereoisomers and tautomers are new.

A = a monocyclic or bicyclic ring (preferably aryl, cycloalkyl, cycloalkenyl, heteroaryl or heterocycle);

R1 = H, alkenyl, alkoxyalkyl, alkoxycarbonylalkyl, alkylcarbonylalkyl, alkylsulfanylalkyl, alkylsulfinylalkyl, alkylsulfonylalkyl, alkynyl, arylsulfanylalkyl, carboxyalkyl, cyanoalkyl, cycloalkenyl, cycloalkenylalkyl, cycloalkyl, (cycloalkyl)alkenyl, (cycloalkyl)alkyl, formylalkyl, haloalkoxyalkyl, (halo)alkyl, (hetero)aryl, (hetero)arylalkenyl, (hetero)arylalkyl, (hetero)arylsulfonylalkyl, heterocycle, heterocyclealkenyl, heterocyclealkyl, hydroxyalkyl, nitroalkyl, RaRbN-, RaRb-Nalkyl-, RaRbNC(O)alkyl-, RaRbNC(O)Oalkyl-, RaRbNC(O)NRcalkyl-, RfRgC=N- or RkO- (all optionally mono- to tri-substituted by T);

T = alkenyl, alkynyl, oxo, halo, cyano, nitro, (halo)alkyl, haloalkoxy, (hetero)aryl, heterocycle, (hetero)arylalkyl, alkoxyalkoxyalkyl, -(alkyl)(ORc), -(alkyl)(NRcRe), -**SRc**, -S(O)Rc, -S(O)2Rc, -ORc, -N(Rc)(Re), -C(O)Rc, -C(O)ORc or -C(O)NRcRe;

R2 and R3 = H, alkenyl, alkoxyalkyl, alkoxycarbonyl, alkyl, alkylcarbonyl, (hetero)aryl, (hetero)arylalkyl, (hetero)arylcarbonyl, heterocyclecarbonyl, cyano, halo or RaRbNC(O)- (both optionally mono- or di-substituted by T);

CR2R3 = 5 or 6 membered ring (preferably cycloalkyl, (hetero)aryl or heterocycle (all optionally substituted by (R6)m);

R4 = alkoxy, arylalkoxy, aryloxy, halo, hydroxy, RaRbN-, N3- or ReS- (all optionally mono- or di-substituted by halo, nitro, cyano, -OH, -NH2 or -COOH);

R5 = alkenyl, alkoxy, alkoxyalkoxyalkyl, alkoxyalkyl, alkoxycarbonyl, alkylcarbonyl, alkylsulfonyl, alkynyl, aryl, arylalkyl, arylcarbonyl, aryloxy, aryloxyalkyl, arylalkoxy, arylsulfonyl, formyl, halo, (halo)alkyl, halocarbonyl, heteroaryl, heteroarylalkyl, heteroarylcarbonyl, heterocycle, heterocycle alkyl, heterocyclecarbonyl, hydroxyalkyl, carboxy, cycloalkyl, cyano, nitro, RaRbN-, RaRbNalkyl-, RaRbNC(O)-, RkOC(O)-, RkOalkyl-, RaRbNSO2-, RaRbNSO2C(O)-, RaRbNSO2alkyl- or ORk (all optionally mono- to tri-substituted by T);

R6 = T;

Ra and Rb = T1, alkylsulfonyl, arylcarbonyl, arylsulfonyl, cycloalkylalkenyl, heteroarylcarbonyl, heteroarylsulfonylalkyl, heterocyclealkenyl, heterocyclecarbonyl, heteroarylsulfonyl, RcRdN-, RcRdNC(O)alkyl-, RcRdNalkylC(O)- or RcRdNC(O)N(Re)alkyl- (all optionally mono- or di-substituted by T);

T1 = H, alkenyl, alkoxyalkyl, alkoxycarbonyl, alkoxycarbonylalkyl, alkyl, alkylcarbonyl, alkylcarbonylalkyl, alkylsulfanylalkyl, alkylsulfonylalkyl, aryl, arylalkyl, arylsulfonylalkyl, carboxyalkyl, cyanoalkyl, cycloalkenyl, cycloalkenylalkyl, cycloalkyl, cycloalkylalkyl, formylalkyl, haloalkyl, heteroaryl, heteroarylalkenyl, heteroarylalkyl, heterocycle, heterocyclealkyl, hydroxyalkyl, nitroalkyl, RcRdNalkyl-, RcRdNC(O)- or RcRdNC(O)Oalkyl-;

Rc and Rd = H, -NH2 -N(H)alkyl, alkenyl, alkynyl, (cyclo)alkyl, cycloalkylalkyl, arylalkyl, (hetero)aryl or heterocycle;

NRcRd = 4 - 6 membered heterocycle (optionally mono- to tri-substituted by T);

Re = H, alkenyl, or (cyclo)alkyl;

Rf and Rg = H, alkenyl, aryl, arylalkyl, (cyclo)alkyl, cycloalkylalkyl, cycloalkenyl, heterocycle, heterocyclealkyl, heteroaryl or heteroarylalkyl;

CRfRg = 4 - 7 membered ring (preferably cycloalkyl, cycloalkenyl or heterocycle);

Rk = T1 or haloalkoxyalkyl (both optionally mono- to tri-substituted by T);

m and n = 0 - 4.

Provided that: CR2R3 is a phenyl ring, and R1 is H, alkenyl, alkynyl, alkoxyalkyl, alkylsulfanylalkyl, alkylsulfinylalkyl, alkylsulfonylalkyl, cyanoalkyl, (halo)alkyl, hydroxyalkyl, arylalkyl, arylalkenyl, cycloalkyl, cycloalkylalkyl, cycloalkylalkenyl, cycloalkylalkynyl, heterocyclealkyl, heterocycloalkenyl, heteroarylalkyl, heteroarylalkenyl or (hetero)aryl; then A is other than phenyl.

ACTIVITY - Virucide; Antiinflammatory; Hepatotropic.

MECHANISM OF ACTION - RNA virus replication inhibitor; Hepatitis C virus (HCV) RNA dependent RNA polymerase inhibitor; HCV replication inhibitor. (I) Were tested for HCV polymerase inhibition.

Two-fold serial dilutions of (I) were incubated with 20 mM Tris-HCl, pH 7.65, 5 mM MgCl2, 50 mM NaCl, 1 mM dithiothreitol, 1 mM ethylene diamine tetraacetic acid (EDTA), 300 micro M GTP and 150 - 300 nM NSSB (HCV strain 1B (J4), AF054247) for 15 minutes at room temperature. The reaction was initiated by the addition of 20 micro M CTP, 20 micro M ATP and 1 micro M 3H-UTP (10 mCi/ micro M), 150 mM template RNA and 0.4 U/ micro l RNase inhibitor, and allowed to proceed for 2 - 4 hours at room temperature. The reaction was terminated by the addition of 1 volume of 4 mM spermine in 10 mM Tris-HCl pH 8 and 1 mM EDTA. After incubation for at least 15 minutes at room temperature, the precipitated RNA was filtered, washed, counted in a scintillation counter, and IC50 was determined. (I) Showed an IC50 of 0.003 - 500 micro M.

USE - For inhibiting the replication of an RNA-containing virus; and for treating or preventing infection caused by an RNA-containing virus (e.g. hepatitis C virus and symptoms of HCV infection (such as cirrhosis and inflammation of liver) (claimed).

ADVANTAGE - The compounds are free of adverse side effects associated with prior art compounds such as flu-like symptoms, leukopenia, thrombocytopenia and depression; associated with interferon, and anemia associated with ribavirin. The compounds are also effective against HCV genotype 1.

Dwg.0/0

L85 ANSWER 20 OF 73 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN

AB WO2003103610 A UPAB: 20040202

NOVELTY - Treatment of a patient infected with a retrovirus or **hepadnavirus** involves administration of a mappicine analog or its salt.

DETAILED DESCRIPTION - Treatment of a patient infected with a retrovirus or **hepadnavirus** involves administration of a mappicine analog of formula (I) or its salt.

Z = -CHOR1R2 or -C(O)R2;

R1 = H, alkyl, aryl, -OC(O)ORa or -C(O)Rb;

Ra = alkyl;

Rb = alkyl, aryl, alkoxy, amino, alkylamino, dialkylamino, aryl, diarylamino or arylalkyl amino;

R2 = alkyl, aryl or arylalkyl;

R3 = H, alkyl, hydroxyalkyl or aryl;

R4 - R8 = H, alkyl, alkenyl, alkynyl, alkoxy, aryloxy, acyloxy, perfluoroalkyl, F, Cl, Br, carbamoyloxy, OH, nitro, cyano, azido, formyl, hydrazine, NR'Rm, T, -OC(O)ORa, -C(O)Rb, -**SRc**, S(O)Rc, S(O)2Rc or (CH2)nSiRdReRf;

T = haloalkyl, cyanoalkyl, azidoalkyl, hydrazinoalkyl, hydroxyalkyl,

alkoxyalkyl, aminoalkyl, alkylaminoalkyl, dialkylaminoalkyl, aryl aminoalkyl, diarylaminoalkyl or arylalkyl aminoalkyl;
R', Rm = H, alkyl, aryl, arylalkyl or -C(O)Rb;
Rc = H, -C(O)Rb, alkyl or aryl;
n = 0 - 10;
Rd - Rf = 1-10C alkyl, 2-10C alkenyl, 2-10C alkynyl, aryl or T; and
R4+R5, R5+R6, R6+R7 and R7+R8 = a chain of 3 or 4 groups selected from CH, CH2, O, S, N, NH, N-alkyl and N-aryl.
ACTIVITY - Anti-HIV; Antiinflammatory; Hepatotropic; Virucide.
MECHANISM OF ACTION - Retroviral reverse transcriptase inhibitor; **Hepadnaviral** reverse transcriptase inhibitor (preferably RNA-dependent DNA polymerase inhibitor and enzyme RNase H inhibitor). 2-tert-Butyloxycarbonylamino-7-(1-hydroxypropyl)-8-methyl-12-trimethylsilanyl-11H-indolizino(1,2-b)quinolin-9-one (AG-2M) was tested in vitro for inhibitory activity against HIV-1 RNase H according to methods described in Inhibition of the Ribonuclease H and DNA Polymerase Activities of HIV-1 Reverse Transcriptase by N-(4-tert-butylbenzoyl)-2-hydroxy-1-naphthaldehyde Hydrazone, Biochemistry 1997, 36, 3179-3185. AG-2M showed IC50 value of 8 micro M.
USE - For treating a patient infected with a retrovirus e.g. HIV or **hepadnavirus** e.g. human **hepatitis B virus** (claimed). The retrovirus infections also include HIV-1, HIV-2, T-cell leukemia virus (HTLV-1 and HTLV-2), feline immunodeficiency virus, feline leukemia virus, bovine immunodeficiency virus, bovine leukemia virus, equine infectious anemia virus, caprine arthritis-encephalitis virus and Rous sarcoma virus infection of chicken.
ADVANTAGE - In contrast to the highly toxic prior art compounds, the mappicine analogs are capable of inhibiting HIV-1 replication in cultured cells and show little toxicity to cells providing potential therapeutic utility.
Dwg.0/4

L85 ANSWER 22 OF 73 MEDLINE on STN DUPLICATE 5
AB We have previously shown that transactivation-proficient hepatitis virus B X protein (**HBx**) protects Hep 3B cells from transforming growth factor-beta (TGF-beta)-induced apoptosis via activation of the phosphatidylinositol 3-kinase (PI 3-kinase)/Akt signaling pathway. This work further investigated how **HBx** activates PI 3-kinase. **Src** activity was elevated in Hep 3B cells following expression of transactivation-proficient **HBx** or **HBx**-GFP fusion proteins. The **Src** family kinase inhibitor PP2 and C-terminal **Src** kinase (Csk) both alleviated **HBx**-mediated PI 3-kinase activation and protection from TGF-beta-induced apoptosis. Therefore, **HBx** activated a survival signal by linking **Src** to PI 3-kinase. Systemic subcellular fractionation and membrane flotation assays indicated that approximately 1.5% of ectopically expressed HBxGFP was associated with periplasmic membrane where **Src** was located. However, neither nucleus-targeted nor periplasmic membrane-targeted HBxGFP was able to upregulate **Src** activity or to augment PI 3-kinase survival signaling pathway.

L85 ANSWER 23 OF 73 MEDLINE on STN DUPLICATE 6
AB Human **hepatitis B virus** (**HBV**) **HBx** protein is a multifunctional protein that activates cellular signaling pathways and is thought to be essential for viral infection. Woodchuck **HBV** mutants that lack **HBx** are unable to replicate in vivo or are severely impaired. **HBV** replication in HepG2 cells, a human hepatoblastoma cell line, is stimulated 5- to 10-fold by **HBx** protein. We have utilized the HepG2, **HBx**-dependent **HBV** replication system to study the effects of activators and inhibitors of cytosolic calcium and tyrosine kinase signaling pathways on viral replication. By transfecting either a wild-type **HBV** genome or an **HBV** genome that does not express **HBx** and then treating transfected cells with activators

or inhibitors of signaling pathways, we identified compounds that either impair wild-type **HBV** replication or rescue **HBx** -deficient **HBV** replication. Geldanamycin or herbimycin A, tyrosine kinase inhibitors, blocked **HBV** replication. Derivatives of cyclosporine, i.e., cyclosporine A, cyclosporine H, and SDZ NIM811, which block cytosolic calcium signaling and specifically the mitochondrial permeability transition pore (SDZ NIM811), also impaired **HBV** replication. Treatment of cells with compounds that increase cytosolic calcium levels by a variety of mechanisms rescued replication of an **HBx**-deficient **HBV** mutant. Transcription of viral RNA and production of viral capsids were only minimally affected by these treatments. These results define a functional signaling circuit for **HBV** replication that includes calcium signaling and activation of cytosolic signaling pathways involving **Src** kinases, and they suggest that these pathways are stimulated by **HBx** acting on the mitochondrial transition pore.

L85 ANSWER 32 OF 73 MEDLINE on STN DUPLICATE 11

AB **Hepatitis B virus** X protein (pX) is implicated in hepatocarcinogenesis by an unknown mechanism. Employing a cellular model linked to pX-mediated transformation, we investigated the role of the previously reported Stat3 activation by pX in hepatocyte transformation. Our model is composed of a differentiated hepatocyte (AML12) 3pX-1 cell line that undergoes pX-dependent transformation and a dedifferentiated hepatocyte (AML12) 4pX-1 cell line that does not exhibit transformation by pX. We report that pX-dependent Stat3 activation occurs only in non-pX-transforming 4pX-1 cells and conclude that Stat3 activation is not linked to pX-mediated transformation. Maximum Stat3 transactivation requires Ser727 phosphorylation, mediated by mitogenic pathway activation. Employing dominant negative mutants and inhibitors of mitogenic pathways, we demonstrate that maximum, pX-dependent Stat3 transactivation is inhibited by the p38 mitogen-activated protein kinase (MAPK)-specific inhibitor SB 203580. Using transient-transreporter and in vitro kinase assays, we demonstrate for the first time that pX activates the p38 MAPK pathway only in 4pX-1 cells. pX-mediated Stat3 and p38 MAPK activation is Ca(2+) and c-**Src** dependent, in agreement with the established cellular action of pX. Importantly, pX-dependent activation of p38 MAPK inactivates Cdc25C by phosphorylation of Ser216, thus initiating activation of the G(2)/M checkpoint, resulting in 4pX-1 cell growth retardation. Interestingly, pX expression in the less differentiated hepatocyte 4pX-1 cells activates signaling pathways known to be active in regenerating hepatocytes. These results suggest that pX expression in the infected liver effects distinct mitogenic pathway activation in less differentiated versus differentiated hepatocytes.

L85 ANSWER 35 OF 73 MEDLINE on STN

AB BACKGROUND/AIMS: **Hepatitis B virus** ( **HBV**) is the etiological factor for hepatocellular carcinoma (HCC). Numerous evidence has indicated a link between chronic infection with **HBV** and the development of HCC. Among the four proteins encoded by **HBV**, **Hepatitis B virus** X gene( **HBx**), best characterized as a transcriptional transactivator, gained attention owing to its presumptive role in oncogenesis. Further, **HBx** has been shown to stimulate signal transduction pathways such as Ras-MAPK pathway, NF-kappa B, and **Src** kinase. The pleiotropic events caused by **HBx** may be the key to understanding the **HBV**-mediated oncogenicity. However, the specific roles of **HBx** in oncogenesis remain largely elusive. To explore the role of **HBx** in hepatocarcinogenesis, we examined the deregulation of host genes induced by **HBx** expression. METHODS: **HBx** was ectopically expressed in HepG2 cells using a recombinant adenovirus to transiently express **HBx.** Gene expression profiling of **HBx** was conducted on cDNA microarrays that contained 1,028 cDNAs. RESULTS: A number of oncogenes and genes that are involved in cell growth,

DNA repair, cell cycle regulation, and cell motility were deregulated by **HBx**. CONCLUSIONS: Theses results suggest that **HBx** regulates transcription in a way that contributes to the proliferation of hepatocytes, a probable early event of HCC.

L85 ANSWER 39 OF 73 MEDLINE on STN DUPLICATE 14
AB Numerous studies have demonstrated that the **hepatitis B virus HBx** protein stimulates signal transduction pathways and may bind to certain transcription factors, particularly the cyclic AMP response element binding protein, CREB. **HBx** has also been shown to promote early cell cycle progression, possibly by functionally replacing the TATA-binding protein-associated factor 250 (TAF(II)250), a transcriptional coactivator, and/or by stimulating cytoplasmic signal transduction pathways. To understand the basis for early cell cycle progression mediated by **HBx**, we characterized the molecular mechanism by which **HBx** promotes deregulation of the G0 and G1 cell cycle checkpoints in growth-arrested cells. We demonstrate that TAF(II)250 is absolutely required for **HBx** activation of the cyclin A promoter and for promotion of early cell cycle transit from G0 through G1. Thus, **HBx** does not functionally replace TAF(II)250 for transcriptional activity or for cell cycle progression, in contrast to a previous report. Instead, **HBx** is shown to activate the cyclin A promoter, induce cyclin A-cyclin-dependent kinase 2 complexes, and promote cycling of growth-arrested cells into G1 through a pathway involving activation of **Src** tyrosine kinases. **HBx** stimulation of **Src** kinases and cyclin gene expression was found to force growth-arrested cells to transit through G1 but to stall at the junction with S phase, which may be important for viral replication.

L85 ANSWER 40 OF 73 MEDLINE on STN DUPLICATE 15
AB Chronic **hepatitis B virus** infection is strongly associated with the development of hepatocellular carcinoma (HCC). Epithelial tumors are frequently characterized by loss of cadherin expression or function. Cadherin-dependent adhesion prevents the acquisition of a migratory and invasive phenotype, and loss of its function is itself enough for the progression from adenoma to carcinoma. The **HBx** protein of **hepatitis B virus** is thought to contribute to the development of the carcinoma, however, its role in the oncogenic and metastatic processes is far from being fully understood. We report herein the ability of **HBx** to disrupt intercellular adhesion in three different cell lines stably transfected with an inducible **HBx** expression vector. The linkage between the actin cytoskeleton and cadherin complex, which is essential for its function, is disrupted in the presence of **HBx**, as indicated by detergent solubility and immunoprecipitation experiments. In addition, beta-catenin was tyrosine phosphorylated in **HBx**-expressing cells. Inhibition of the **src** family of tyrosine kinases resulted in the prevention of the disruption of adherens junctions. These results suggest that **HBx** is able to disrupt intercellular adhesion in a **src**-dependent manner, and provide a novel mechanism by which **HBx** may contribute to the development of HCC.

L85 ANSWER 41 OF 73 MEDLINE on STN DUPLICATE 16
AB **Hepatitis B virus** (**HBV**) infects more than 300 million people and is a leading cause of liver cancer and disease. The **HBV HBx** protein is essential for infection; **HBx** activation of **Src** is important for **HBV** DNA replication. In our study, **HBx** activated cytosolic calcium-dependent proline-rich tyrosine kinase-2 (Pyk2), a **Src** kinase activator. **HBx** activation of **HBV** DNA replication was blocked by inhibiting Pyk2 or calcium signaling mediated by mitochondrial calcium channels, which suggests that **HBx** targets mitochondrial calcium regulation. Reagents that

increased cytosolic calcium substituted for **HBx** protein in **HBV** DNA replication. Thus, alteration of cytosolic calcium was a fundamental requirement for **HBV** replication and was mediated by **HBx** protein.

L85 ANSWER 51 OF 73 MEDLINE on STN DUPLICATE 18
AB Chronic infection by **hepatitis B virus** is a leading cause of human liver cancer and liver disease. The **hepatitis B virus HBx** protein is a regulatory factor that is essential for virus infection in mammals and is implicated in development of liver cancer and liver disease. Among the reported activities of **HBx** is the ability to stimulate **Src** tyrosine kinases, Ras-GTPases and transcriptional activation. We now demonstrate that **HBx** activation of **Src** tyrosine kinases, but not Ras, promotes a high level of viral replication in cell culture. **HBx** is shown to stimulate reverse transcription of the viral pregenomic mRNA into genomic DNA through a **Src**-mediated pathway in tissue culture cells. Targeted inhibition of **Src** tyrosine kinase activity, mutational inactivation of the **HBx** gene or retargeting of **HBx** to the nucleus to abolish cytoplasmic signal transduction activity, are shown to impair viral reverse transcription strongly. These studies implicate **HBx** stimulation of the **Src** family of tyrosine kinases in stimulation of viral polymerase activity.

L85 ANSWER 53 OF 73 MEDLINE on STN DUPLICATE 20
AB **Hepatitis B virus** is a causative agent of hepatocellular carcinoma, and in the course of tumorigenesis, the X-gene product (**HBx**) is known to play important roles. Here, we investigated the transforming potential of **HBx** by conventional focus formation assay in NIH3T3 cells. Cells were cotransfected with the **HBx** expression plasmid along with other oncogenes including Ha-ras, v-**src**, v-myc, v-fos, and E1a. Unexpectedly, the introduction of **HBx** completely abrogated the focus-forming ability of all five tested oncogenes. In addition, the cotransfection of Bcl-2, an apoptosis inhibitor, reversed the **HBx**-mediated inhibition of focus formation, suggesting that the observed repression of focus formation by **HBx** is through the induction of apoptosis. Next, to test unequivocally whether **HBx** induces apoptosis in liver cells, we established stable Chang liver cell lines expressing **HBx** under the control of a tetracycline-inducible promoter. Induction of **HBx** in these cells in the presence of 1% calf serum resulted in typical apoptosis phenomena such as DNA fragmentation, nuclear condensation, and fragmentation. Based on these results, we propose that **HBx** sensitizes liver cells to apoptosis upon **hepatitis B virus** infection, contributing to the development of hepatitis and the subsequent generation of hepatocellular carcinoma.

L85 ANSWER 58 OF 73 MEDLINE on STN DUPLICATE 21
AB The **HBx** protein of **hepatitis B virus** (**HBV**) is a small transcriptional transactivator that is essential for infection by the mammalian **hepadnaviruses** and is thought to be a cofactor in **HBV**-mediated liver cancer. **HBx** stimulates signal transduction pathways by acting in the cytoplasm, which accounts for many but not all of its transcriptional activities. Studies have shown that **HBx** protein activates Ras and downstream Ras signaling pathways including Raf, mitogen-activated protein (MAP) kinase kinase kinase (MEK), and MAP kinases. In this study, we investigated the mechanism of activation of Ras by **HBx** because it has been found to be central to the ability of **HBx** protein to stimulate transcription and to release growth arrest in quiescent cells. In contrast to the transient but strong stimulation of Ras typical of autocrine factors, activation of Ras by **HBx** protein was found to be constitutive but moderate. **HBx** induced

the association of Ras upstream activating proteins Shc, Grb2, and Sos and stimulated GTP loading onto Ras, but without directly participating in complex formation. Instead, **HBx** is shown to stimulate Ras-activating proteins by functioning as an intracellular cytoplasmic activator of the **Src** family of tyrosine kinases, which can signal to Ras. **HBx** protein stimulated c-**Src** and **Fyn** kinases for a prolonged time. Activation of **Src** is shown to be indispensable for a number of **HBx** activities, including activation of Ras and the Ras-Raf-MAP kinase pathway and stimulation of transcription mediated by transcription factor AP-1. Importantly, **HBx** protein expressed in cultured cells during **HBV** replication is shown to activate the Ras signaling pathway. Mechanisms by which **HBx** protein might activate **Src** kinases are discussed.

L85 ANSWER 64 OF 73 MEDLINE on STN

AB In the current study we sought to elucidate the molecular mechanisms which might contribute to hepatocarcinogenesis in a **hepatitis B virus** (**HBV**) envelope transgenic mouse model in which chronic hepatocellular injury and inflammation lead to regenerative hyperplasia and eventually to the development of chromosomal abnormalities and hepatocellular carcinoma (HCC), thereby reiterating many of the pathophysiological events that occur prior to the development of HCC in chronic **HBV** infection in humans. We have previously demonstrated that **HBV** envelope gene expression is decreased in regenerating hepatocytes and preneoplastic nodules early in the disease process and that expression of alpha-fetoprotein and the multidrug transporter gene mdr-III is activated in the tumors that develop in this model, but not prior to tumor development. In the current study, we examined the structure and expression of a large panel of dominant acting oncogenes and tumor suppressor genes in the liver at all stages of the disease process in order to determine the extent to which they contribute to hepatocarcinogenesis in these transgenic mice. To our surprise, no changes were observed in the structure or function of any of these genes, many of which are commonly activated in other rodent models of hepatocarcinogenesis but rarely activated in human HCC. These findings suggest that the **HBV** transgenic mouse model is different from most other rodent models of hepatocarcinogenesis and that it may relate more closely to the events involved in **HBV**-induced human hepatocarcinogenesis, where generalized chromosomal abnormalities are common, while structural and functional changes in most of the commonly studied positive-acting oncogenes examined herein are not. Since p53 and RB mutations have recently been reported to be late events in human hepatocarcinogenesis, the structural integrity of the RB locus and the absence of p53 mutations in the **HBV** transgenic mouse model suggest that they may represent a relatively early stage of hepatocellular tumorigenesis and that further manipulation of this model is warranted in order to more fully reproduce the molecular-genetic events that characterize **HBV**-induced HCC in humans.

=> log y

| COST IN U.S. DOLLARS | SINCE FILE ENTRY | TOTAL SESSION |
|---|---|---|
| FULL ESTIMATED COST | 158.41 | 158.68 |

STN INTERNATIONAL LOGOFF AT 11:54:07 ON 14 JUN 2005

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
|---|---|---|---|---|---|---|
| L1 | 12597 | hepadnavir$ or whv or hbv or hepatitis b virus | US-PGPUB; USPAT | ADJ | OFF | 2005/06/14 09:59 |
| L2 | 131882 | src or fyn or lck or yes or lyn or blk or fgr or hck | US-PGPUB; USPAT | ADJ | OFF | 2005/06/14 10:00 |
| L3 | 94 | 1 same 2 | US-PGPUB; USPAT | ADJ | OFF | 2005/06/14 10:13 |
| L4 | 39 | 1 near10 2 | US-PGPUB; USPAT | ADJ | OFF | 2005/06/14 10:00 |
| L5 | 190 | hbx | US-PGPUB; USPAT | OR | OFF | 2005/06/14 10:12 |
| L6 | 30 | 5 same 2 | US-PGPUB; USPAT | ADJ | OFF | 2005/06/14 10:13 |

| Ref # | Hits | Search Query | DBs | Default Operator | Plurals | Time Stamp |
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| L1 | 12597 | hepadnavir$ or whv or hbv or hepatitis b virus | US-PGPUB; USPAT | ADJ | OFF | 2005/06/14 09:59 |
| L2 | 131882 | src or fyn or lck or yes or lyn or blk or fgr or hck | US-PGPUB; USPAT | ADJ | OFF | 2005/06/14 10:00 |
| L3 | 94 | 1 same 2 | US-PGPUB; USPAT | ADJ | OFF | 2005/06/14 10:00 |
| L4 | 39 | 1 near10 2 | US-PGPUB; USPAT | ADJ | OFF | 2005/06/14 10:00 |

PGPUB-DOCUMENT-NUMBER: 20050049246

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20050049246 A1

TITLE: Inhibitors of Src and Lck protein kinases

PUBLICATION-DATE: March 3, 2005

INVENTOR-INFORMATION:

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APPL-NO: 10/ 728113

DATE FILED: December 4, 2003

RELATED-US-APPL-DATA:

child 10728113 A1 20031204

parent division-of 10171895 20020614 US GRANTED

parent-patent 6689778 US

non-provisional-of-provisional 60302969 20010703 US

US-CL-CURRENT: 514/227.8, 514/235.5 , 514/252.19 , 514/275 , 544/122 , 544/295 , 544/331 , 544/60

ABSTRACT:

The present invention provides compounds of formula I: 1 or a pharmaceutically acceptable derivative thereof, wherein A-B is N--O or O--N and G, R.sup.1, R.sup.2, R.sup.3, and R.sup.4 are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of Src and Lck kinase. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application No.60/302.,969-filed Jul. 3, 2001, the contents of which are incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (9):
[0008] Src also plays a role in the replication of hepatitis B virus. The

virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J., 18, 5019, (1999) and Klein et al., Mol. Cell. Biol., 17, 6427 (1997).

PGPUB-DOCUMENT-NUMBER: 20040220200

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040220200 A1

TITLE: Compositions useful as protein kinase inhibitors

PUBLICATION-DATE: November 4, 2004

INVENTOR-INFORMATION:

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| Hale, Michael R. | Bedford | MA | US | |
| Moon, Young-Choon | Belle Meade | NJ | US | |


APPL-NO: 10/ 798766

DATE FILED: March 11, 2004

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60454405 20030313 US

US-CL-CURRENT: 514/269, 514/275 , 514/343 , 544/331 , 546/276.4

ABSTRACT:

The present invention relates to compounds useful of inhibitors of protein kinases. The invention also provides pharmaceutically acceptable compositions comprising said compounds and methods of using the compositions in the treatment of various disease, conditions, or disorders.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application Ser. No. 60/454,405, filed Mar. 13, 2003 the contents of which are incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (49):
[0048] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J. 1999, 18, 5019, and Klein et al., Mol. Cell. Biol. 1997, 17, 6427.

PGPUB-DOCUMENT-NUMBER: 20040214817

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040214817 A1

TITLE: Diaminotriazoles useful as inhibitors of protein kinases

PUBLICATION-DATE: October 28, 2004

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Pierce, Albert C. | Cambridge | MA | US | |
| Amost, Michael | North Andover | MA | US | |
| Davies, Robert J. | Arlington | MA | US | |
| Forster, Cornelia J. | Pelham | NH | US | |
| Galullo, Vincent | South Grafton | MA | US | |
| Grey, Ronald JR. | Cambridge | MA | US | |
| Ledeboer, Mark | Acton | MA | US | |
| Tian, Shi-Kai | Waltham | MA | US | |
| Xu, Jinwang | Framingham | MA | US | |
| Binch, Hayley | Harwell | MA | GB | |
| Ledford, Brian | Attleboro | MA | US | |
| Messersmith, David | Somerville | MA | US | |
| Nanthakumar, Suganthi | Newton | MA | US | |
| Jayaraj, Andrew | Needham | CA | US | |
| Henkel, Greg | Carlsbad | MA | US | |
| Salituro, Francesco G. | Marlboro | MA | US | |
| Wang, Jian | Newton | | US | |

APPL-NO: 10/ 715111

DATE FILED: November 17, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60426681 20021115 US

non-provisional-of-provisional 60447705 20030211 US

US-CL-CURRENT: 514/217.09, 514/227.5 , 514/235.8 , 514/254.05 , 514/326 , 514/383 , 544/132 , 544/366 , 544/60 , 546/208 , 548/264.8

ABSTRACT:

The present invention relates to inhibitors of protein kinases. The invention also provides pharmaceutical compositions comprising the compounds of the invention and methods of using the compositions in the treatment of various disorders.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority under 35 U.S.C. .sctn.119 to U.S. Provisional Application No. 60/426,681, filed Nov. 15, 2002, entitled "Compositions Useful as Inhibitors of Protein Kinases, and 60/447,705, filed Feb. 11, 2003, entitled "Compositions Useful as Inhibitors of Protein Kinases", and the entire contents of each of these applications is hereby

incorporated by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (21):
[0020] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J. 1999, 18, 5019, and Klein et al., Mol. Cell. Biol. 1997, 17, 6427.

PGPUB-DOCUMENT-NUMBER: 20040192696

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040192696 A1

TITLE: Compositions useful as inhibitors of protein kinases

PUBLICATION-DATE: September 30, 2004

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Green, Jeremy | Burlington | MA | US | |
| Grey, Ronald JR. | Cambridge | MA | US | |
| Pierce, Albert C. | Cambridge | MA | US | |


APPL-NO: 10/ 738956

DATE FILED: December 17, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60435124 20021218 US

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | DOC-ID | APPL-DATE |
|---|---|---|---|
| WO | PCT/US03/39990 | 2003WO-PCT/US03/39990 | December 17, 2003 |

US-CL-CURRENT: 514/248, 514/227.8 , 514/234.5 , 544/117 , 544/236 , 544/60

ABSTRACT:

The present invention provides a compound of formula (I): 1
or a pharmaceutically acceptable salt thereof. These compounds are inhibitors of protein kinases, particularly inhibitors of PIM-1, CDK-2, GSK-3, and SRC mammalian protein kinases. The invention also provides pharmaceutically acceptable compositions comprising the compounds of the invention and methods of utilizing those compounds and compositions in the treatment of various protein kinase mediated disorders.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority under 35 U.S.C. .sctn.119 to U.S. Provisional Application No.: 60/435,124, filed Dec. 18, 2002, entitled "Compositions Useful as Inhibitors of Protein Kinases, the entire contents of which is hereby incorporated by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (26):
[0025] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J., 18, 5019, (1999) and Klein et al., Mol. Cell. Biol., 17, 6427 (1997).

PGPUB-DOCUMENT-NUMBER: 20040126771

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040126771 A1

TITLE: PRNA chimera

PUBLICATION-DATE: July 1, 2004

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Guo, Peixuan | West Lafayette | IN | US | |
| Hoeprich, Stephen M. | North Canton | OH | US | |
| Shu, Dan | West Lafayette | IN | US | |


APPL-NO: 10/ 373612

DATE FILED: February 24, 2003

RELATED-US-APPL-DATA:

child 10373612 A1 20030224

parent continuation-in-part-of PCT/US01/26333 20010823 US PENDING

non-provisional-of-provisional 60433697 20021216 US

non-provisional-of-provisional 60227393 20000823 US

US-CL-CURRENT: 435/6, 530/350 , 536/23.1

ABSTRACT:

A circularly permuted chimeric pRNA molecule carrying a stabilized biologically active RNA, such as a ribozyme.

[0001] This application claims the benefit of U.S. provisional patent application Ser. No. 60/433,697, filed Dec. 16, 2002, and also is a continuation-in-part patent application of PCT/US01/26333, filed Aug. 23, 2001, which claims the benefit of U.S. provisional patent application Ser. No. 60/227,393, filed Aug. 23, 2000, each of which patent applications is incorporated herein by reference in its entirety.

---------- KWIC ---------

Detail Description Table CWU - DETL (6):

6TABLE 1 Plasmids, oligos and PCR products used for the assay of ribozyme activities Target or Contains Name Function Promoter Purpose pRNA cpDNA3A Circularly SP.sub.6 Production Yes (plasmid) permutated pRNA, of cpRNA in vitro cpDNAT.sub.7 Circularly SP.sub.6 Construction Yes (plasmid) permutated pRNA, of chimeric in vitro ribozme pRNA- Ribozyme, in vitro T.sub.7 HBV polyA Yes RzA (plasmid) pRzA Ribozyme, in vitro T.sub.7 HBV polyA No (plasmid) pTZS Substrate, in vitro T.sub.7 HBV polyA No (plasmid) pRNA- Ribozyme, CMV HBV polyA Yes CRzA tissue culture (plasmid) pCRzA Ribozyme, CMV HBV polyA

No (plasmid) tissue culture pCdRzA Disabled ribozyme, CMV HBV polyA No (plasmid) tissue culture p3.6II HBV HBV polyA No (plasmid) genomic RNAs, tissue culture U7 Substrate, in vitro T.sub.7 U7 No (oligos) RzU7 Ribozyme, in vitro T.sub.7 U7 No (oligos) PRNA- Ribozyme, in vitro T.sub.7 U7 Yes RzU7 (PCR) 12-LOX Substrate, in vitro T.sub.7 12-LOX No (oligos) Rz12lox Ribozyme, in vitro T.sub.7 12-LOX No (oligos) PRNA- Ribozyme, in vitro T.sub.7 12-LOX Yes Rz12lox (PCR)

PGPUB-DOCUMENT-NUMBER: 20040106615

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040106615 A1

TITLE: Protein kinase inhibitors and uses thereof

PUBLICATION-DATE: June 3, 2004

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Cochran, John | Marshfield | MA | US | |
| Green, Jeremy | Burlington | MA | US | |
| Hale, Michael R. | Bedford | MA | US | |
| Ledford, Brian | Attleboro | MA | US | |
| Maltais, Francois | Tewksbury | MA | US | |
| Nanthakumar, Suganthini | Newton | MA | US | |

APPL-NO: 10/ 639784

DATE FILED: August 12, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60403256 20020814 US

non-provisional-of-provisional 60416802 20021008 US

US-CL-CURRENT: 514/242, 514/247 , 514/252.03 , 514/275 , 544/183 , 544/238 , 544/331

ABSTRACT:

Described herein are compounds that are useful as protein kinase inhibitors having the formulae I and V: 1
or a pharmaceutically acceptable salt thereof, wherein Ring B, Z.sup.1, Z.sup.2, U, T, m, n, p, Q, Q', R.sup.1, R.sup.2, R.sup.x, R.sup.3, and R.sup.6 are as defined herein. These compounds, and pharmaceutically acceptable compositions thereof, are useful for treating or lessening the severity of a variety of disorders, including stroke, inflammatory disorders, autoimmune diseases such as SLE lupus and psoriasis, proliferative disorders such as cancer, and conditions associated with organ transplantation.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Applications 60/403,256 filed Aug. 14, 2002 and 60/416,802 filed Oct. 8, 2002, the contents of which are incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (79):
[0078] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J. 1999, 18, 5019, and Klein et al., Mol. Cell. Biol. 1997, 17, 6427.

PGPUB-DOCUMENT-NUMBER: 20040097531

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040097531 A1

TITLE: Inhibitors of c-Jun N-terminal kinases (JNK) and other protein kinases

PUBLICATION-DATE: May 20, 2004

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Ledeboer, Mark | Acton | MA | US | |
| Wang, Jian | Boston | MA | US | |
| Moon, Young Choon | Belle Mead | NJ | US | |

APPL-NO: 10/ 616560

DATE FILED: July 9, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60395202 20020709 US

US-CL-CURRENT: 514/275, 514/341 , 514/342 , 544/331 , 546/270.4 , 546/271.4 , 546/272.7

ABSTRACT:

The present invention provides compounds of formula I: 1
or a pharmaceutically acceptable derivative thereof, wherein R.sup.1, R.sup.2, A, G, and W are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of JNK, a mammalian protein kinase involved cell proliferation, cell death and response to extracellular stimuli, Lck, Src, and Aurora kinases. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application 60/395,202, filed Jul. 9, 2002, which is hereby incorporated by reference in its entirety.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (19):
[0018] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus [Klein et al., EMBO J., 18:5019, (1999) and Klein et al., Mol. Cell. Biol., 17:6427 (1997)].

PGPUB-DOCUMENT-NUMBER: 20040077663

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040077663 A1

TITLE: Thienopyrimidine-based inhibitors of the src family

PUBLICATION-DATE: April 22, 2004

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Benish, Michele A. | Pearland | TX | US | |
| Lawless, Michael | St. Charles | MD | US | |
| Budde, Raymond J. | Bellaire | TX | US | |


APPL-NO: 10/ 399816

DATE FILED: October 6, 2003

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | DOC-ID | APPL-DATE |
|---|---|---|---|
| US | 09694145 | 2000US-09694145 | October 23, 2000 |

PCT-DATA:
APPL-NO: PCT/US01/50198
DATE-FILED: Oct 23, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 514/260.1, 544/280

ABSTRACT:

Various thienopyrimidine-based analog compounds are able to selectively inhibit the Src family of tyrosine kinases. These compounds are useful in the treatment of various diseases including hyperproliferative diseases, hematologic diseases, osteoporosis, neurological diseases, autoimmune diseases, allergic/immunological diseases, or viral infections.

---------- KWIC --------

Summary of Invention Paragraph - BSTX (12):

[0012] Herpesviridae, papovaviridae, and retroviridae have been shown to interact with non-receptor tyrosine kinases and use them as signaling intermediates. The HIV-1 Nef protein interacts with members of the Src family of tyrosine kinases. Nef mediates downregulation of CD4 membrane expression, modification of T-cell activation pathways, and increases virus infectivity (Collette et al., 1997). The HBx protein of the hepatitis B virus is essential for infection by hepadnaviruses and activates Ras by activating the Src family of tyrosine kinases. The activation of Ras is necessary for the ability of the HBx protein to stimulate transcription and release growth arrest in quiescent cells (Klein and Schneider, 1997). Activity of the Src family of tyrosine kinases is altered by association with viral proteins such as mouse and hamster

polyomavirus middle-T antigens, Epstein-Barr virus LMP2A, and herpesvirus saimiri Tip (Dunant and Ballmer-Hofer, 1997).

Detail Description Paragraph - DETX (270):
[0334] Klein N P and Schneider R J. Activation of Src Family Kinases by Hepatitis B Virus HBx Protein and Coupled Signaling to Ras. Mol Cell Biol 17:6427-6436, 1997.

PGPUB-DOCUMENT-NUMBER: 20040023963

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040023963 A1

TITLE: Inhibitors of c-Jun N-terminal kinases (JNK) and other protein kinases

PUBLICATION-DATE: February 5, 2004

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Cao, Jingrong | Newton | MA | US | |
| Green, Jeremy | Burlington | MA | US | |
| Moon, Young-Choon | Lexington | MA | US | |
| Wang, Jian | Boston | MA | US | |
| Ledeboer, Mark | Acton | MA | US | |
| Harrington, Edmund | South Boston | MA | US | |
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APPL-NO: 10/ 437666

DATE FILED: May 14, 2003

RELATED-US-APPL-DATA:

child 10437666 A1 20030514

parent division-of 10121035 20020410 US PENDING

non-provisional-of-provisional 60283621 20010413 US

non-provisional-of-provisional 60329440 20011015 US

non-provisional-of-provisional 60292974 20010523 US

US-CL-CURRENT: 514/242, 514/227.8 , 514/235.8 , 514/252.01 , 514/275 , 544/112 , 544/182 , 544/238 , 544/331 , 544/60

ABSTRACT:

The present invention provides compounds of formula I: 1
or a pharmaceutically acceptable derivative o thereof, wherein A, B, R.sup.a, R.sup.1, R.sup.2, R.sup.3, and R.sup.4 are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of JNK, a mammalian protein kinase involved cell proliferation, cell death and response to extracellular stimuli; Lck and Src kinase. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of priority to co-pending U.S. provisional applications No. 60/283,621 filed Apr. 13, 2001, No. 60/329,440 filed Oct. 14, 2001 and No. 60/292,974 filed May 23, 2001.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (19):

[0018] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J., 18, 5019, (1999) and Klein et al., Mol. Cell. Biol., 17, 6427 (1997).

PGPUB-DOCUMENT-NUMBER: 20040009981

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040009981 A1

TITLE: Compositions useful as inhibitors of protein kinases

PUBLICATION-DATE: January 15, 2004

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
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| Charrier, Jean-Damien | Grove Wantage | | GB | |
| Everitt, Simon | Beaconsfield | | GB | |
| Golec, Julian M.C. | Ashbury | | GB | |
| Kay, David | Purton | | GB | |
| Knegtel, Ronald | Abingdon | | GB | |
| Miller, Andrew | Upton | | GB | |
| Pierard, Francoise | Drayton | | GB | |


APPL-NO: 10/ 389259

DATE FILED: March 14, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60364864 20020315 US

US-CL-CURRENT: 514/242, 514/260.1 , 514/263.2 , 514/265.1 , 514/266.23 , 514/269 , 544/182 , 544/262 , 544/277 , 544/280 , 544/284 , 544/317

ABSTRACT:

The present invention relates to compounds useful as inhibitors of protein kinases. The invention also provides pharmaceutically acceptable compositions comprising said compounds and methods of using the compositions in the treatment of various disease, conditions, or disorders.

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application 60/364,864 filed Mar. 15, 2002 the entirety of which is incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (18):
[0017] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J. 1999, 18, 5019, and Klein et al., Mol. Cell. Biol. 1997, 17, 6427.

PGPUB-DOCUMENT-NUMBER: 20040009974

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040009974 A1

TITLE: Compositions useful as inhibitors of protein kinases

PUBLICATION-DATE: January 15, 2004


INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Bebbington, David | Newbury | | GB | |
| Binch, Hayley | Harwell | | GB | |
| Charrier, Jean-Damien | Grove Wantage | | FR | |
| Everitt, Simon | Beaconsfield | | GB | |
| Golec, Julian M.C. | Ashbury | | GB | |
| Kay, David | Purton | | GB | |
| Knegtel, Ronald | Abingdon | | DK | |
| Miller, Andrew | Upton | | GB | |
| Pierard, Francoise | Drayton | | BE | |



APPL-NO: 10/ 389296

DATE FILED: March 14, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60365003 20020315 US

US-CL-CURRENT: 514/227.8, 514/235.8 , 514/242 , 514/252.19 , 514/269 , 544/123 , 544/182 , 544/295 , 544/317 , 544/60



ABSTRACT:

The present invention relates to compounds useful of inhibitors of protein kinases. The invention also provides pharmaceutically acceptable compositions comprising said compounds and methods of using the compositions in the treatment of various disease, conditions, or disorders.


---------- KWIC ---------

Summary of Invention Paragraph - BSTX (20):
[0017] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J. 1999, 18, 5019, and Klein et al., Mol. Cell. Biol. 1997, 17, 6427.

PGPUB-DOCUMENT-NUMBER: 20040002496

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040002496 A1

TITLE: Compositions useful as inhibitors of protein kinases

PUBLICATION-DATE: January 1, 2004

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Bebbington, David | Newbury | | GB | |
| Binch, Hayley | Harwell | | GB | |
| Charrier, Jean-Damien | Grove Wantage | | GB | |
| Everitt, Simon | Beaconsfield | | GB | |
| Golec, Julian M. C. | Ashbury | | GB | |
| Kay, David | Wiltshire | | GB | |
| Knegtel, Ronald | Abingdon | | GB | |
| Miller, Andrew | Upton | | GB | |
| Pierard, Francoise | Drayton | | GB | |


APPL-NO: 10/ 389709

DATE FILED: March 14, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60364840 20020315 US

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | DOC-ID | APPL-DATE |
|---|---|---|---|
| WO | PCT/US03/07904 | 2003WO-PCT/US03/07904 | March 14, 2003 |

US-CL-CURRENT: 514/245, 514/227.8 , 514/238.8 , 514/252.19 , 514/275 , 544/113 , 544/122 , 544/198 , 544/209 , 544/295 , 544/324 , 544/60

ABSTRACT:

The present invention relates to compounds useful as inhibitors of protein kinases. The invention also provides pharmaceutically acceptable compositions comprising said compounds and methods of using the compositions in the treatment of various disease, conditions, or disorders.

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application No. 60/364,840 filed Mar. 15, 2002 the entirety of which is incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (18):

[0017] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J. 1999, 18, 5019, and Klein et

al., Mol. Cell. Biol. 1997, 17, 6427.

PGPUB-DOCUMENT-NUMBER: 20030225073

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030225073 A1

TITLE: Compositions useful as inhibitors of protein kinases

PUBLICATION-DATE: December 4, 2003


INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Bebbington, David | Newbury | MA | GB | |
| Binch, Hayley | Harwell | | GB | |
| Charrier, Jean-Damien | Grove Wantage | | GB | |
| Everitt, Simon | Beaconsfield | | GB | |
| Golec, Julian M.C. | Ashbury | | GB | |
| Kay, David | Purton | | GB | |
| Knegtel, Ronald | Abingdon | | GB | |
| Miller, Andrew | Upton | | GB | |
| Pierard, Francoise | Drayton | | GB | |
| Pierce, Albert C. | Cambridge | | US | |



APPL-NO: 10/ 389707

DATE FILED: March 14, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60364842 20020315 US

US-CL-CURRENT: 514/227.8, 514/235.8 , 514/241 , 514/242 , 544/112 , 544/113 , 544/182 , 544/209 , 544/60



ABSTRACT:

The present invention relates to compounds useful as inhibitors of protein kinases. The invention also provides pharmaceutically acceptable compositions comprising said compounds and methods of using the compositions in the treatment of various disease, conditions, or disorders.


CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application No. 60/364,842 filed Mar. 15, 2002 the entirety of which is incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (18):

[0017] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J. 1999, 18, 5019, and Klein et al., Mol. Cell. Biol. 1997, 17, 6427.

PGPUB-DOCUMENT-NUMBER: 20030207873

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030207873 A1

TITLE: Inhibitors of Src and other protein kinases

PUBLICATION-DATE: November 6, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Harrington, Edmund | South Boston | MA | US | |

APPL-NO: 10/ 119890

DATE FILED: April 10, 2002

US-CL-CURRENT: 514/227.8, 514/235.8 , 514/241 , 514/252.19 , 514/275 , 544/122 , 544/212 , 544/295 , 544/331 , 544/60

ABSTRACT:

The present invention provides compounds of formula I: 1
wherein A is N or CR, and R.sup.1, G, and R.sup.2, are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of Src mammalian protein kinase involved in cell proliferation, cell death and response to extracellular stimuli. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application 60/282,935 filed Apr. 10, 2001, the contents of which are incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (9):
[0008] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J., 18, 5019, (1999) and Klein et al., Mol. Cell. Biol., 17, 6427 (1997).

PGPUB-DOCUMENT-NUMBER: 20030199676

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030199676 A1

TITLE: Universal procedure for refolding recombinant proteins

PUBLICATION-DATE: October 23, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---|---|---|---|---|
| Lin, Xinli | Piedmont | CA | US | |

APPL-NO: 10/ 420044

DATE FILED: April 17, 2003

RELATED-US-APPL-DATA:

child 10420044 A1 20030417

parent continuation-of 09752878 20001228 US GRANTED

parent-patent 6583268 US

non-provisional-of-provisional 60177836 20000125 US

non-provisional-of-provisional 60178368 20000127 US

non-provisional-of-provisional 60210292 20000608 US

non-provisional-of-provisional 60210306 20000608 US

US-CL-CURRENT: 530/350, 530/412

ABSTRACT:

A universal folding method that has been demonstrated to be effective in refolding a variety of very different proteins expressed in bacteria as inclusion bodies has been developed. Representative proteins that can be dissolved and refolded in biologically active form, with the native structure, are shown in Table I. The method has two key steps to unfold and then refold the proteins expressed in the inclusion bodies. The first step is to raise the pH of the protein solution in the presence of denaturing agents to pH greater than 9, preferably 10. The protein solution may be maintained at the elevated pH for a period of up to about 24 hours, or the pH immediately decreased slowly, in increments of about 0.2 pH units/24 hours, until the solution reaches a pH of about 8.0, or both steps used. In the preferred embodiment, purified inclusion bodies are dissolved in 8 M urea, 0.1 M Tris, 1 mM glycine, 1 mM EDTA, 10 mM beta-mercaptoethanol, 10 mM dithiothreitol (DTT), 1 mM redued glutathion (GSH), 0.1 mM oxidized glutathion (GSSG), pH 10. The absorbance at 280 nm (OD280) of the protein solution is 5.0. This solution is rapidly diluted into 20 volumes of 20 mM Tris base. The resulting solutin is adjusted to pH 9.0 with 1 M HCl and is kept at 4.degree. C. for 24 hr. The pH is adjusted to pH 8.8 and the solution is kept at 4.degree. C. for another 24 hrs. This process is repeated until the pH is adjusted to 8.0. After 24 hr at pH 8.0, the refolded proteins can be concentrated by ultrafiltration and

applied to a gel filtration column for purification.

---------- KWIC --------

Summary of Invention - Table CWU - BSTL (1):

1TABLE I Expression, refolding, and purification of different proteins from E. coli Name From Organism Refold Purification Ref. Pepsinogen Full-length Porcine Yes Yes Lin, et al, 1989 Pepsinogen N and C Porcine Yes Yes Lin, et al, 1992 Domain Lin, et al, 1993 Rhizopus- full-length Fungus Yes Yes Chen, et al, 1991 Pepsinogen Lin, et al, 1992 Thermopsin full-length Archae No No None Thermopsin fusion Archae Yes Partial Lin, Liu, Tang, 92 Cathepsin D full-length human No No None low yield Pregnancy full-length Bovine No No None Specific Ant Ovine HIV protease full-length HIV Yes Yes Lin, et al, 1995 Ermolief, et al, 97 SAP full-length Yeast Yes Yes Lin, et al, 1993 Koelsch, et al, 98 Streptokinase full-length bacteria Yes Yes Wang, et al, 1998 Plasminogen cat-domain human Yes Yes Wang, et al, 2000 Cadosin A full-length plant Yes Yes Faro, et al, 1999 Napsin 1 full-length human No No Koelsch, et al, 00 Memapsin 2 full-length human Yes Yes Lin, et al, 2000 Memapsin 1 full-length human Yes Yes PreS partial HBV Yes Yes unc-76 full-length C. elegans Yes Yes odc-1 full-length C. elegans Yes Yes ceh-10 full-length C. elegans Yes Yes ppp-1 full-length C. elegans No No References for Table 1: Lin, X, Wong, R.N.S., and Tang, J. (1989) "Synthesis, purification, and active site mutagenesis of recombinant porcine pepsinogen". J. Biol. Chem. 264:4482-4489. Lin, X.L., Lin, Y.-Z., Koelsch, G., Gustchina, A., Wlodawer, A., and Tang, J. (1992) "Enzymic activities of two-chain pepsinogen, two-chain pepsin, and the amino-terminal lobe of pepsinogen". J. Biol. Chem. 267:17257-17263. Lin, X., Loy, J.A., Sussman, F., and Tang, J. (1993) "Conformational instability of the N- and C-terminal lobes of porcine pepsin in neutral and alkaline solutions". Prol. Sci. 2:1383-1390. Chen, Z., Koelsch, G., Han, H.-P., Wang, X.-J., Lin, X., Hartsuck, J.A., and Tang, J. (1991) "Recombinant rhizopuspepsinogen", J. Biol. Chem. 266:11718-11725. Lin, Y,-Z., Fusek, M., Lin, X.L., Hartsuck, J.A., Kezdy, F.J., and Tang, J. (1992) "pH Dependence of kineticparameters of pepsin, rhizopuspepsin, and their active-site hydrogen bond mutants". J. Biol. Chem. 267:18413-18418. Lin, X.L., Liu, M.T., and Tang, J. (1992) "Heterologous expression of thermopsin, a heat stable acid proteinase". Enzyme Microb. Technol. 14:696-701. Lin, Y.-Z., Lin, X., Hong, L., Foundling, S., Heinrikson, R.L., Thaisrivongs, S., Leelamanit, W., Raterman, D., Shah, M., Dunn, B.M., and Tang, J. (1995) "Effect of point mutations on the kinetics and the inhibition of human immunodeficiency virus type 1 protease: relationship to drug resistance". Biochemistry 34:1143-1152. Ermolieff, J., Lin, X., and Tang, J. (1997) Kinetic properties of saquinavir-resistant mutants of human immunodeficiency virus type 1 protease and their implications in drug resistance in vivo. Biochemistry 36:12364-12370. Lin, X., Tang, J., Koelsch, G., Monod, M., and Foundling, S. (1993) "Recombinant canditropsin, an extracellular aspartic protease from yeast Candida tropicalis". J. Biol. Chem. 268_20143-20147. Koelsch, G., Tang, J., Monod, M., Foundling, S.I., Lin, X. (1998) "Primary substrate specificities of secreted aspartic proteases of Candida albicans". Adv. Exp. Med. Biol. 436:335-333. Wang, X., Lin, X., Lowy, J.A., Tang, J., Zhang, X.C. (1998) "Crystal structure of the catalytic domain of human plasmin complexed with streptokinase", Science. 281:1662-1665. Wang, X., Terzyan, S., Tang, J., Loy, J., Lin, X., and Zhang, X. (2000) "Human plasminogen catalytic domain undergoes a novel conformational change upon activation" J. Mol. Biol. (in press). Faro, C., Ramalho-Santos, M., Vieira, M., Mendes, A., Simoes, I., Andrade, R., Verissimo, P., Lin, X., Tang, J., Pires, E. (1999) "Cloning and Characterization of cDNA Encoding Cardosin A, an RGD-containing Plant Aspartic Proteinase," J. Biol. Chem. 274(40):28724-28729. Lin, X., Koelsch, G., Wu, S., Downs, D., Dashti, A., and Tang, J. (2000) "Human aspartic protease

memapsin 2 cleaves the .beta.-secretase site of .beta.-amyloid precursor protein. Proc. Natl. Aca. Sci. 97(4):1456-1460. Ghosh, A.K., Shin, D., Downs, D., Koelsch, G., Lin, X., Ermolieff, J., Tang, J. (2000) "Design of potent inhibitors form human brain memapsin 2 (.beta.-secretase)" J. Amer. Chem. Soc., 122:3522-3523.

PGPUB-DOCUMENT-NUMBER: 20030171389

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030171389 A1

TITLE: Inhibitors of Src and Lck protein kinases

PUBLICATION-DATE: September 11, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 171895

DATE FILED: June 14, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60302969 20010703 US

US-CL-CURRENT: 514/275, 544/331

ABSTRACT:

The present invention provides compounds of formula I: 1
or a pharmaceutically acceptable derivative thereof, wherein A-B is N--O or O--N and G, R.sup.1, R.sup.2, R.sup.3, and R.sup.4 are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of Src and Lck kinase. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application No. 60/302,969 filed Jul. 3, 2001, the contents of which are incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (9):
[0008] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J., 18, 5019, (1999) and Klein et al., Mol.Cell. Biol., 17, 6427 (1997).

PGPUB-DOCUMENT-NUMBER: 20030152916

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030152916 A1

TITLE: Detection of HIV

PUBLICATION-DATE: August 14, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 244490

DATE FILED: September 16, 2002

RELATED-US-APPL-DATA:

child 10244490 A1 20020916

parent continuation-of 09168947 19981008 US PENDING

child 09168947 19981008 US

parent continuation-in-part-of 08469067 19950606 US GRANTED

parent-patent 5824518 US

child 08469067 19950606 US

parent continuation-of 07550837 19900710 US GRANTED

parent-patent 5480784 US

child 07550837 19900710 US

parent continuation-in-part-of 07379501 19890711 US ABANDONED

US-CL-CURRENT: 435/5, 435/6 , 435/91.2 , 536/24.3

ABSTRACT:

The present invention relates to oligonucleotides for use in amplifying and detecting HIV nucleic acid in a sample.

[0001] This application is a continuation-in-part of U.S. Ser. No. 08/469,067 filed Jun. 6, 1995, which is a continuation of U.S. Ser. No. 07/550,837, filed Jul. 10, 1990, both are hereby incorporated by reference herein in their entirety (including the drawings).

---------- KWIC --------

Detail Description Table CWU - DETL (6):
7TABLE 6 Preliminary Procedure II System. Reaction 1 2 3 4 5 6 7 M13L(-) No No Yes Yes Yes Yes Yes T7pro(+) Yes Yes No Yes No Yes Yes T7pro(-) Yes No No No Yes Yes No HBV(-) Pr No Yes No No No No Yes Probe Relative Light Units (RLU's) Probe(+) 862 744 762 1089 2577 96221 30501 Probe(-) 473 420 483 3038 1080 15171 14863

PGPUB-DOCUMENT-NUMBER: 20030152912

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030152912 A1

TITLE: Multiple viral replicon culture systems

PUBLICATION-DATE: August 14, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
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APPL-NO: 10/ 060941

DATE FILED: January 29, 2002

US-CL-CURRENT: 435/5, 435/235.1 , 435/7.1 , 435/91.1 , 435/91.2 , 435/91.3 , 514/44

ABSTRACT:

Methods and compositions are provided for screening candidate antiviral agents using cells containing subgenomic viral replication systems such as replicons and minigenomes. The methods involve the simultaneous assay of more than one subgenomic viral replication system. Compositions useful for these methods are also provided.

---------- KWIC ---------

Detail Description Table CWU - DETL (1):

1TABLE 1 Viral replicons for antiviral screening Infec- tious Noncytopathic Family Virus (common names) clone replicon Togaviridae Sindbis yes yes Venezuela encephalitis virus yes possible Rubella yes possible Picornaviridae Poliovirus yes yes Coxsackirus yes possible Enterovirus yes possible Hepatitis A yes possible Flaviviridae Yellow fever yes yes Dengue fever yes possible West Nile virus possible Japanese Encephalitis virus yes yes Hepatitis C virus yes yes Tick-born encephalitis virus possible (TBE) Astroviridae Astrovirus yes possible Rhabdoviridae Rabies virus yes possible Orthomyxoviridae Influenza virus A yes possible Influenza virus B possible Paramyxoviridae Respiratory syncytial virus yes possible (RSV) Measles yes possible Mumps yes possible Filoviridae Ebola yes possible Marburg possible Bunyaviridae La Crosse virus possible California encephalitis virus yes possible Hantaan virus possible Crimean-Congo possible Rift Valley fever possible Arenaviridae Lassa fever possible Argentine Hemorrhagic fever possible Bolivian Hemorrhagic fever possible Reoviridae Colorado tick fever Hepadanviridae Hepatitis B virus yes yes Papillomaviridae Human papilloma virus yes yes Polyomaviridae JC virus yes possible BK virus yes possible Herpeviruses Herpes simplex virus type yes yes one (HSV-1) Herpes simplex virus type yes possible two (HSV-2) Epstein-Barr virus (EBV) yes yes Human cytomegalovirus yes possible (HCMV) Varicella-zoster virus (VZV) yes possible Human herpes virus type poss- possible six (HHV6) ible Human herpes virus type possi- possible seven (HHV7) ible Human herpes virus type poss-

possible eight (HHV8) ible Adenoviridae Human adenovirus yes possible Retrovirus Human immunodeficiency yes possible virus type one (HIV-1) Human immunodeficiency yes possible virus type two (HIV-2) Human t-cell leukemia virus yes possible type one (HTLV-1) Human t-cell leukemia virus yes possible type two (HTLV-2) Parvoviridae Human parvovirus yes possible Adeno-associated virus yes yes

PGPUB-DOCUMENT-NUMBER: 20030144309

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030144309 A1

TITLE: Inhibitors of Src and other protein kinases

PUBLICATION-DATE: July 31, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
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APPL-NO: 10/ 146984

DATE FILED: May 16, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60291340 20010516 US

US-CL-CURRENT: 514/275, 514/227.8 , 514/235.8 , 514/252.19 , 514/253.09 , 514/341 , 544/122 , 544/124 , 544/295 , 544/331 , 544/360 , 544/60 , 546/275.4

ABSTRACT:

The present invention provides compounds of formula I: 1 wherein A is N or CR, and G, R.sup.1, R.sup.2 and R.sup.3 are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of Src mammalian protein kinase involved in cell proliferation, cell death and response to extracellular stimuli. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application 60/291,340 filed May 16, 2001, the contents of which are incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (9):

[0008] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J., 18, 5019, (1999) and Klein et al., Mol.Cell. Biol., 17, 6427 (1997).

PGPUB-DOCUMENT-NUMBER: 20030096816

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030096816 A1

TITLE: Inhibitors of c-Jun N-terminal kinases (JNK) and other protein kinases

PUBLICATION-DATE: May 22, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 121035

DATE FILED: April 10, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60283621 20010413 US

non-provisional-of-provisional 60329440 20011015 US

non-provisional-of-provisional 60292974 20010523 US

US-CL-CURRENT: 514/242, 514/252.01 , 514/275 , 544/182 , 544/238 , 544/331

ABSTRACT:

The present invention provides compounds of formula I: 1
or a pharmaceutically acceptable derivative o thereof, wherein A, B, R.sup.a, R.sup.1, R.sup.2, R.sup.3, and R.sup.4 are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of JNK, a mammalian protein kinase involved cell proliferation, cell death and response to extracellular stimuli; Lck and Src kinase. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of priority to co-pending U.S. provisional applications 60/283,621 filed Apr. 13, 2001, 60/329,440 filed Oct. 14, 2001 and 60/292,974 filed May 23, 2001.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (19):

[0018] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J., 18, 5019, (1999) and Klein et al., Mol.Cell. Biol., 17, 6427 (1997).

PGPUB-DOCUMENT-NUMBER: 20030087922

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030087922 A1

TITLE: Inhibitors of c-Jun N-terminal kinases (JNK) and other protein kinases

PUBLICATION-DATE: May 8, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 109070

DATE FILED: March 28, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60279961 20010329 US

US-CL-CURRENT: 514/275, 544/330 , 544/331

ABSTRACT:

The present invention provide a compound of formula I or II: 1
or a pharmaceutically acceptable derivative thereof, wherein R.sup.1, R.sup.2, R.sup.3, and R.sup.4 are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of JNK, a mammalian protein kinase involved cell proliferation, cell death and response to extracellular stimuli; and Src-family kinases, especially Src and Lck kinases. These compounds are also inhibitors of GSK3 and CDK2 kinases. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application No. 60/279,961 filed Mar. 29, 2001, the contents of which is incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (20):
[0019] Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J., 18, 5019, (1999) and Klein et al., Mol. Cell. Biol., 17, 6427 (1997).

PGPUB-DOCUMENT-NUMBER: 20030032596

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030032596 A1

TITLE: Inhibition of the Src kinase family pathway as a method of treating HBV infection and hepatocellular carcinoma

PUBLICATION-DATE: February 13, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 196344

DATE FILED: July 15, 2002

RELATED-US-APPL-DATA:

child 10196344 A1 20020715

parent continuation-of 08874430 19970613 US GRANTED

parent-patent 6420338 US

US-CL-CURRENT: 514/12, 514/262.1 , 514/44

ABSTRACT:

The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target HBx mediated activation of Src kinase, members of the Src tyrosine kinase family and components of the Src kinase family signal transduction pathways for the treatment of HBV infection and related disorders and diseases, such as HCC. The invention further relates to pharmaceutical compositions for the treatment of HBV infection targeted to HBx and its essential activities required to sustain HBV replication. The invention is based, in part, on the Applicants' discovery that activation of Src kinase signaling cascades play a fundamental role in mammalian hepadnavirus replication. Applicants have demonstrated that HBx mediates activation of the Src family of kinases and that this activation is a critical function provided by HBx for mammalian hepadnavirus replication.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):
The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target HBx mediated activation of Src kinase, members of the Src tyrosine kinase family and components of the Src kinase family signal transduction pathways for the treatment of HBV infection and related disorders and diseases, such as HCC. The invention further relates to pharmaceutical compositions for the treatment of HBV infection targeted to HBx and its essential activities required to sustain HBV replication. The invention is based, in part, on the Applicants' discovery that activation of Src kinase signaling cascades play a fundamental role in mammalian hepadnavirus

replication. Applicants have demonstrated that HBx mediates activation of the Src family of kinases and that this activation is a critical function provided by HBx for mammalian hepadnavirus replication.

Title - TTL (1):
Inhibition of the Src kinase family pathway as a method of treating HBV infection and hepatocellular carcinoma

Summary of Invention Paragraph - BSTX (2):
[0001] The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target Src family kinases and components of the Src kinase family signal transduction pathways, including HBx activation of Src kinase family signal transduction pathways for the treatment and prevention of hepatitis B virus (HBV) infection and hepatocellular carcinoma (HCC). The invention also relates to screening assays to identify potential antiviral agents which target HBx-mediated activation of Src kinase signaling cascades for the treatment of HBV.

Summary of Invention Paragraph - BSTX (17):
[0014] The present invention relates to the treatment and prevention of HBV infection by targeting activation of the Src family of kinases. The present invention also relates to compounds which inhibit HBx-mediated activation of the Src family of kinases as well as the downstream components of the Src kinase signaling cascade for the treatment of HBV infection.

Summary of Invention Paragraph - BSTX (18):
[0015] The invention is based, in part on the Applicants' surprising discovery that activation of a Src kinase signaling cascade is a critical function provided by HBx for mammalian hepadnavirus replication. The Applicants have shown that Src kinases are also activated during HBV infection of cultured cells and that this activation is an essential function of the viral HBx protein. Thus, the Applicants have demonstrated that the HBx-mediated activation of the Src kinase signaling cascade plays a fundamental role in mammalian hepadnavirus replication.

Summary of Invention Paragraph - BSTX (19):
[0016] The Applicants have demonstrated that HBx mediated activation of Src kinase signaling cascade is an effective target for HBV anti-viral agents since activation of this pathway is essential for HBV replication. Therefore, targeting HBx for the treatment of HBV should result in a highly specific and efficacious method of blocking HBV replication. The Src family of kinases, although host cell gene products, are only activated in proliferating or differentiating cells, and in cells infected by many DNA and tumor viruses. Therefore, targeting the Src family of kinases for the treatment of HBV infection should result in a therapeutic with a high degree of efficacy and sufficient specificity with side effects no more toxic than chemotherapeutics currently used to treat cancer.

Summary of Invention Paragraph - BSTX (20):
[0017] The present invention encompasses a variety of techniques and compounds to target the activities of HBx essential for HBV replication. In particular, these include, but are not limited to HBx-mediated activation of the Src kinase family signal transduction pathways for the treatment and prevention of HBV infection. The invention encompasses the use of known Src inhibitors to treat HBV infection. Examples of such specific inhibitors include, but not limited to: Src specific tyrosine kinase inhibitors, such as CsK, tyrphostin-derived inhibitors, derivatives of benzylidenemalonitrile, pyrazolopyrimidine PP1, and microbial agents, such as angelmicin B; and competitive inhibitors, such as small phosphotyrosine containing ligands. The

invention also encompasses the use of known HBx inhibitors for the treatment of HBV, including, but not limited to, antisense RNAs directed to HBx. The present invention also relates to the use of inhibitors of downstream effectors of Src kinases, including but not limited to, cytoplasmic factors, such as Ras, Raf, focal adhesion kinase (FAK) and MAPK, and nuclear factors, such as Myc and cyclin-dependent kinases.

Summary of Invention Paragraph - BSTX (21):
[0018] In another embodiment of the present invention gene therapy approaches, including dominant-negative mutants, antisense molecules and SELEX RNAs targeted to block Src kinase or HBx gene expression, may be used as a method to treat and prevent HBV infection and HCC. In yet another embodiment of the invention, upstream and downstream components and effectors of the Src kinase family signaling cascade may be targeted by gene therapy approaches to inhibit HBV infection.

Summary of Invention Paragraph - BSTX (22):
[0019] The present invention further relates to screening assays to identify compounds which inhibit HBx-mediated activation of the Src kinase signaling pathway and may be used to treat HBV infection and diseases and disorders associated with HBV infection.

Summary of Invention Paragraph - BSTX (23):
[0020] The invention is illustrated by way of working examples which demonstrate that HBx mediates activation of a Src kinase signaling cascade and that activation of this signaling cascade is an essential function of HBx required to sustain HBV replication. The working examples of the present invention further demonstrate the ability of inhibitors of the Src kinase signaling cascade to inhibit HBV replication.

Summary of Invention Paragraph - BSTX (26):
[0023] As used herein, the term "target protein" refers to those proteins which correspond to Src kinase or members of the Src kinase family or components of the Src kinase signaling pathway or proteins encoded by the HBV genome, including HBx.

Brief Description of Drawings Paragraph - DRTX (7):
[0036] FIG. 6. Woodchuck Hepatitis B Virus (WHV) HBx protein (WHx) activates a Src-Ras signaling cascade during WHV replication in cultured cells. Chang cells were cotransfected with 20 .mu.g pcWHV or wtWHV with 8 .mu.g of either dominant-negative Ras, kinase inactive (dominant-negative) Src, or Csk plasmids. Eighteen hours post-transfection, cells were serum-starved in 0.5% CS for 20 hours, MAP kinase (ERK-2) was immunoprecipitated from equal amounts of cell lysates and pellets were subjected to an in vitro MBP kinase assay.

Brief Description of Drawings Paragraph - DRTX (8):
[0037] FIG. 7. WHx requires activation of Src family kinase for WHV replication. Chang cells were co-transfected with 20 .mu.g PCWHV, wtWHV, wtWHV and RasDN (dominant-negative) (10 .mu.g), or wtWHV and Csk (10 .mu.g). Three days post-transfection viral core-associated DNA was isolated, purified, and subjected to Southern blot analysis using a full-length .sup.32P-labeled WHV genomic probe.

Detail Description Paragraph - DETX (2):
[0038] The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target HBx-mediated activation of Src kinase, members of the Src tyrosine kinase family and components of the Src

kinase family signal transduction pathways for the treatment of HBV infection and related disorders and diseases, such as HCC. The invention further relates to pharmaceutical compositions for the treatment of HBV-infection targeted to HBx and its essential activities required to sustain HBV replication.

Detail Description Paragraph - DETX (3):
[0039] The invention is based, in part, on the Applicants' discovery that activation of Src kinase signaling cascades plays a fundamental role in mammalian hepadnavirus replication. Applicants have demonstrated that HBx mediates activation of the Src family of kinases and that this activation is a critical function provided by HBx for mammalian hepadnavirus replication.

Detail Description Paragraph - DETX (6):
[0042] The present invention relates to cell-based and animal model based screening assays to identify novel anti-HBV agents which target HBx and its interaction and/or activation of cellular components of the Src kinase signaling cascade. In addition, the present invention relates to screening assays to identify novel antiviral agents which inhibit HBx mediated activation of Src kinase and/or downstream effectors of the Src kinase signaling cascade, such as the nuclear factor, Myc. A variety of protocols and techniques may be utilized to screen for agents which interfere with and/or inhibit the interaction and/or activation of the Src kinase signaling cascade by HBx.

Detail Description Paragraph - DETX (9):
5.1 THE ROLE OF HBx MEDIATED SRC KINASE ACTIVATION IN HBV-INFECTION AND ITS USE AS A TARGET FOR INTERVENTION

Detail Description Paragraph - DETX (10):
[0045] The present invention is based, in part, on the Applicants' surprising discovery that (1) HBx acts as an intracellular, cytoplasmic activator of the Src family of nonreceptor tyrosine kinases; (2) HBx stimulates tyrosine kinase activity of the Src family kinase members, including c-Src and c-Fyn; and (3) inhibition of Src activity by the expression of a Src inhibitor, e.g., the Csk protein, results in the dramatic inhibition of HBV replication. This discovery is exemplified in the in Sections 6, 7, 8 and 9 infra, which demonstrate that activation of Src kinase and the Src kinase signaling cascade is required to sustain HBV replication, and that inhibition of Src kinase dramatically inhibits HBV replication.

Detail Description Paragraph - DETX (14):
[0049] Applicants have further demonstrated that the expression a Src inhibitors, i.e., Csk protein, or dominant-negative Src or Fyn proteins resulted in the inhibition of HBx activation of downstream components of Src kinase signaling cascade. Applicants have also shown that the expression of Src dominant-negative mutants, such as Csk, inhibited the ability of HBx to stimulate activities of the nuclear factor, Myc, including stimulation of cell cycle progression by blocking HBx activation of Src kinase signaling pathways. These findings clearly establish that activation of a Src kinase signaling cascade by HBx has a critical role in the hepadnaviral life cycle.

Detail Description Paragraph - DETX (15):
[0050] HBx mediated activation of Src is required for HBV replication as demonstrated by way of example (Section 9 infra). The Applicants' work demonstrates that an essential component of the requirement of HBx viral replication in cultured cells is its ability to activate Src signaling cascades. HBx activation of a Src signaling cascade plays a critical role in transcriptional upregulation of the viral mRNAs. Inhibition of Src activity by the expression of a Src inhibitor, e.g., the CsK protein, results in the dramatic inhibition of HBV replication. These results illustrate that

activation of Src family kinases has an essential role during HBV replicative life cycles.

Detail Description Paragraph - DETX (16):
[0051] The Applicants' discovery has implicated several targets for effective HBV anti-viral agents. HBV therapies that target the viral gene product HBx should result in a high degree of specificity and efficacy. HBV therapies that target the host cell gene products, the Src family of kinases, should likewise demonstrate specificity and efficacy. Although host cell gene products, the Src family of kinases are active in proliferating cells, such as cancer cells, or in virally infected cells. Therefore, targeting the Src family of kinases for the treatment of HBV infection should result in a high degree of efficacy, and sufficient specificity with side effects no more toxic than chemotherapeutics currently used to treat cancer.

Detail Description Paragraph - DETX (17):
5.2 TREATMENT OF HBV-INFECTION USING INHIBITORS OF HBX MEDIATED SRC ACTIVATION

Detail Description Paragraph - DETX (18):
[0052] The present invention encompasses a variety of therapeutic protocols, methods and compounds to target HBx-mediated activation of the Src kinase signaling cascade for the treatment of HBV. The present invention encompasses all of the compounds described in the subsections below to target HBx-mediated activation of the Src kinase signaling cascade with the proviso that they are not known in the art to be used to treat HBV infection, including, for example, interferon .alpha., interferon .delta., interleukin-1, interleukin-2, immune-active peptides, such as thymosin-alpha, nucleoside analogs, such as vidarabine, fialuridine, lamivuridine, famciclovir, ribavarin, and corticosteroids, such as prednisone and azathioprine.

Detail Description Paragraph - DETX (23):
[0055] A variety of techniques and compositions may be utilized to target Src kinase to inhibit its activity or to inhibit HBx mediated activation of components of the Src kinase mediated signaling cascade, thereby inhibiting HBV replication. Such techniques and compositions may include, but are not limited to, gene therapy approaches, drugs, small organic molecules identified to inhibit Src kinase, Ras, Raf, MAPK kinase, MAPK, c-Myc, cyclin-dependent kinases and/or other downstream effectors of the Src kinase signaling cascade.

Detail Description Paragraph - DETX (24):
[0056] In particular, compounds which may be used in accordance with the present invention to specifically target activation of Src kinase are binding proteins and competing ligands that prevent the intramolecular interaction between the carboxy-terminal phosphorylated tyrosine residue and the SH2 domain located in the amino-terminal half of the molecule and the immediately adjacent SH3 domain (Lin et al., 1993, Oncogene 8:1119-1126). In particular, compounds which may also be used in accordance with the present invention include tyrosine kinase inhibitors which block the activity the Src kinase signaling cascade and therefore would block HBV replication. Examples of such tyrosine kinase inhibitors include, but are not limited to, tyrphostin-derived inhibitors, which are derivatives of benzylidenemalonitrile, have been shown to have strong inhibitory activity of Src (Ramdas et al., 1995, Archives of Biochemistry and Biophysics 323:237-242), pyrazolopyrimidine PP1 (4-amino-5-(4-methylphenyl)-7-(t-butyl) pyrazolo [3,4-d] pyrimidine, a selective inhibitor of the Src family of kinases (Hanke et al., 1996, J. Biol. Chem. 271:695-791) and derivatives thereof. Other examples include microbial agents, such as angelmicin B, a specific inhibitor of Src tyrosine kinase activity, and derivatives thereof (Yokoyama et al., 1996, Leukemia Research

20:491-497), which may also be used to inhibit HBV replication.

Detail Description Paragraph - DETX (25):
[0057] In another embodiment of the present invention, small peptides which compete with larger phosphotyrosine peptides for binding to the Src kinase protein may be used to inhibit the Src kinase signaling cascade, in particular small phosphotyrosine containing peptide ligands, 5 to 6 amino acids, which are able to compete with larger phosphotyrosine-containing peptides and protein ligands for binding to SH2 domains, thereby inhibiting the Src kinase signaling cascade and blocking replication of HBV. Another embodiment of the present invention includes small peptides which correspond to catalytic or enzymatic domains of Src kinase and would compete with Src kinase, inhibiting the activation of downstream components of the Src kinase signaling cascade. Another embodiment includes the use of larger polypeptides that inhibit Src kinase activity including, but not limited to, Csk (carboxyl-terminal Src kinase) which is a specific physiologic inhibitor of Src kinase. Further examples of larger polypeptides that inhibit Src kinase activity include, for example, Src dominant-negative mutants, i.e., Srck-(Barone et al., 1995, Nature 378:509-512) and Fyn dominant-negative mutants (Twamley-Stein et al., 1993, Proc. Natl. Acad. Sci. USA 90:7696-7700), also included are dominant-negative mutants of downstream effectors of the Src kinase signaling cascade, including Ras, Raf, MAPK kinase, MAPK dominant-negative mutants and Myc dominant-negative mutants (Sawyers et al., 1992, Cell 70:901-910).

Detail Description Paragraph - DETX (28):
[0059] In one embodiment of the invention, novel antiviral agents identified by the screening methods of the present invention are used in combination with known therapies to treat HBV infection, for example, IFN, interleukin-1, interleukin-2, immune-active peptides, nucleoside analogs and corticosteriods. The antiviral agents identified by the screening methods of the present invention may also be used in combination with exogenous or endogenous agents which induce IFN expression. In yet another embodiment, inhibitors of Src kinase are used in combination with agents which induce an anti-HBV immune response in order to target two different molecules required in the viral life cycle.

Detail Description Paragraph - DETX (48):
[0076] In yet another specific embodiment, attenuated viruses, such as hepadnaviruses, which have the same tropism as HBV, may be engineered and used for gene therapy in accordance with the present invention. Hepadnaviruses are particularly attractive for use in gene therapy in accordance with the present invention as these viruses will deliver the therapeutic exactly to those cells infected with HBV. Hepadnaviral vectors would be particularly effective for the delivery of nucleic acids targeting components of the Src kinase signaling cascade, thereby avoiding unnecessarily knocking out expression of host genes.

Detail Description Paragraph - DETX (90):
[0112] At least two different assay systems, described in the subsections below, can be designed and used to identify compounds or compositions that modulate HBx-mediated activation of Src kinase signaling cascades and thereby inhibit HBV replication.

Detail Description Paragraph - DETX (91):
[0113] The systems described below may be formulated into kits. To this end, cells expressing HBx and components of the Src kinase signaling cascade, or cells expressing components of the Src kinase signaling cascade which are capable of sustaining HBV replication, or cell lysates thereof can be packaged in a variety of containers, e.g., vials, tubes, microtitre well plates, bottles, and the like. Other reagents can be included in separate containers

and provided with the kit; e.g., positive control samples, negative control samples, buffers, cell culture media, etc.

Detail Description Paragraph - DETX (95):
[0116] Alternately, cell lines which co-express HBx and Src kinase and components of the Src kinase signaling cascade may be genetically engineered to assay for inhibitors of HBx activation of Src. This can be engineered in cell in the absence of HBV replication or in cell lines which support the HBV life cycle as a means of (1) identifying additional factors required to support the HBV life cycle, and (2) as a system to screen test compounds, for their ability to interfere with HBx activation and/ or interaction with the Src kinase, and (3) as a system to screen test compounds for their ability to inhibit Src kinase activity and therefore inhibit HBV replication.

Detail Description Paragraph - DETX (104):
[0125] Alternatively, activation of Src kinase signaling pathways mediated by HBx may be measured by the secretion of mature HBV viral particles into the medium of growing Chang cells. For example, Chang liver cells may be stably transformed with an HBV or WHV pregenome, or with a head-to-tail dimer of either HBV or WHV genomes. The integrated virus will produce and secrete HBV/WHV particles into the medium. As demonstrated by the Applicants, the secretion of viral particles is strongly enhanced by HBx protein activation of Src kinases. If the test compound is effective in inhibiting HBx activation of Src, it will result in reduced secretion of HBV/WHV particles into the medium. The level of particle secreted into the medium can be assayed using commercial ELISA kits to detect the presence of HBV/WHV HBcAg and HBsAg.

Detail Description Paragraph - DETX (110):
[0130] In preferred embodiment of the invention, Src kinase is expressed alone in transgenic Src knock-out mice and a HBV pseudovirus is used to infect the animals. For example, a HBV pseudovirus which contains the HBV virus and an envelope protein from a virus with a natural tropism for murine cells, such as the murine leukemia virus (MLV), is used to bypass internalization of the HBV virus by the murine cells. These murine cells can then support the life cycle of the internalized HBV virus, because they express human Src kinase.

Detail Description Paragraph - DETX (112):
[0132] In yet another embodiment of the animal model screens of the present invention, the effect of test compounds to inhibit HBV replication may be measured indirectly. For example, transgenic mice may be engineered which express (1) the HBx gene product under the control of an inducible promoter, and (2) readout vector which is responsive to Src activation. The readout vector may comprise a reporter gene under the control of a Myc promoter. Such reporter constructs are described in Section 5.5.1 infra. In this assay system, expression of the HBx gene product is induced and the test compound is administered to the mice. The ability of the test compound to inhibit HBx mediated activation of Src kinase and HBV replication is assayed by measuring the reporter gene. Such reporter genes may include but are not limited to chloramphenicol acetyltransferase (CAT), luciferase, GUS, growth hormone, or placental alkaline phosphatase (SEAP). Following exposure of the animal to the test compound, the level of reporter gene expression may be quantitated from the blood or tissue sample to determine the test compound's ability to regulate receptor activity. Alkaline phosphatase assays are particularly useful in the practice of the invention as the enzyme is secreted from the cell. Therefore, tissue culture supernatant may be assayed for secreted alkaline phosphatase. In addition, alkaline phosphatase activity may be measured by calorimetric, bioluminescent or chemilumenscent assays such as those described in Bronstein, I. et al. (1994, Biotechniques 17: 172-177). Such assays provide a simple, sensitive easily detection system for pharmaceutical screening.

Detail Description Paragraph - DETX (161):
9. EXAMPLE: WHV REQUIRES SRC FAMILY KINASES FOR IN VITRO REPLICATION

Detail Description Paragraph - DETX (166):
[0174] Chang cells were co-transfected with plasmids encoding 20 either wtWHV, PCWHV, wtWHV and RasDN, or wtWHV and Csk, intracellular core-associated viral DNA was isolated 3 days post-transfection, and purified viral DNA analyzed by Southern blot hybridization as described above (FIG. 7). Consistent with the previous data, accumulation of viral DNA replicative intermediates was strongly enhanced in cells expressing wtWhV, as compared to cells expressing PCWHV. Coexpression of RasDN protein with wtWHV had no detectable effect on viral replication, and viral DNA was synthesized at near wild-type levels. Expression of the RasDN protein impaired WHV activation of MAP kinase under these same experimental conditions (FIG. 7), indicating that the RasDN protein was able to function during WHV replication. However, these results illustrate the effects of one particular inhibitor of Ras and do not provide an explanation of the mechanism by which activation of Src kinase supports HBV replication. In sharp contrast, co-expression of Csk with wtWHV completely abolished the ability of wtWHV to replicate to high levels. These results demonstrated that an essential component of the requirement of HBx during in vitro viral replication in Chang cells is its ability to activate Src signaling cascades, and that activation of Src family kinases has a critical role during the viral replicative life cycle.

Detail Description Paragraph - DETX (168):
[0176] To assess whether HBx activation of a Src signaling cascade plays an essential role in transcriptional upregulation of the viral mRNAs, Chang cells were cotransfected with wtWHV and either RasDN or Csk plasmids, and the RNA visualized by Northern analysis (FIG. 7, lanes 2 and 3). Co-expression of RasDN with wtWHV only slightly reduced the amount of the RNA species, while co-expression of wtWHV with Csk reduced the RNA level .about.3-5 fold, to the level also observed by expression of pCWHV. To ensure that the decrease in synthesis of WHV RNA by Csk was not the unforseen consequence of Csk inhibition of the CMV promoter (which drives synthesis of the WHV pregenomic RNA), control experiments assessing the effect of Csk on a CMV-.beta.gal reporter were carried out. In comparison with expression of CMV-.beta.gal alone, co-expression of Csk with CMV-.gamma.gal did not significantly alter expression of the .beta.gal protein as measured by its .beta.-galactosidase activity (Sambrook et al, 1989, supra). This control experiment indicates that expression of Csk does not generally inhibit transcription of the CMV promoter, and rules out a non-specific effect of Csk on viral transcription. Therefore, these data suggest that the HBx protein moderately increases the abundance of all the viral transcripts through activation of the Src family of kinases. However, the WHx-induced increase in mRNA abundance (.about.3-5 fold) is much less then the HBx-induced increase in viral DNA synthesis (.about.20-30 fold). Therefore, transcriptional transactivation by HBx does not appear to fully account for the augmentation of viral replication by the HBx protein. The stimulation of Src signaling cascades by HBx must therefore promote WHV replication independent of the effect of WHx on viral transcription. These results illustrate that HBx activates a Src-Ras signaling cascade during viral replication in vitro which is essential for the host cell to sustain HBV replication.

Detail Description Paragraph - DETX (169):
10. EXAMPLE: WHV REQUIRES SRC FAMILY OF KINASES FOR IN VIVO REPLICATION

Detail Description Paragraph - DETX (170):
[0177] The requirement for activation of Src kinase family members in

replication of WHV can be determined in woodchuck infected livers in the following manner. A 2-5 year old woodchuck is experimentally infected using a pooled serum from previous chronic carrier woodchucks. After 2 years of chronic infection, determined by WHsAg ELISA, the infected liver is surgically removed, the liver is perfused as described (Jacob et al., 1994, Exp. Cell Res. 212:42-48), hepatocytes are dispersed by collagenase treatment and plated onto collagen coated dishes in L15 medium supplemented with 5% fetal calf serum, hydrocortisone and insulin. To introduce an inhibitor of Src kinases into primary hepatocytes, the Csk gene is cloned into the left-end of a replication-defective adenovirus vector under the control of the CMV promoter, as described (Doria et al., 1995, EMBO J. 14:4747-4757). Adenovirus vectors infect primary cells and express trans-genes efficiently, whereas it is not possible to transfect such cells at a high rate. Within several days of plating, cells are infected with the Csk-adenovirus vector, medium is replaced with L15 medium lacking insulin and containing reduced serum (between 0.5-2%). Cells are then harvested at 2 and 4 days after introduction of the vector. The medium can be assayed for levels of secreted WHV by ELISA for WHcAg and WHsAg. The level of virus replication can be assayed as described for Chang cells.

Claims Text - CLTX (24):

23. A pharmaceutical formulation for the treatment of HBV infection, comprising a compound that inhibits activation of a Src kinase, mixed with a pharmaceutically acceptable carrier.

Claims Text - CLTX (35):

34. A yeast cell for use in screening agents effective to inhibit HBV infection or replication in a host cell wherein the yeast cell inducibly expresses the Src kinase gene.

Claims Text - CLTX (37):

36. A method for screening for a potential antiviral agent for the treatment of HBV infection comprising, (a) inducing the expression of Src in the yeast cell of claim 34; (b) administering a test compound to the cell; (c) measuring the activation of Src kinase; and (d) determining whether the presence of the test compound reduces the activity of Src kinase, in which test compounds that result in decreased activity of a Src kinase are identified as potential antiviral agents.

PGPUB-DOCUMENT-NUMBER: 20020045191

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020045191 A1

TITLE: Inhibition of the SRC kinase family pathway as a method of treating HBV infection and hepatocellular carcinoma

PUBLICATION-DATE: April 18, 2002

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APPL-NO: 09/ 955006

DATE FILED: September 17, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60232892 20000915 US

US-CL-CURRENT: 435/7.1, 435/15 , 514/262.1 , 514/44 , 514/520 , 514/7 , 536/24.5

ABSTRACT:

The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target HBx mediated activation of Src kinase, members of the Src tyrosine kinase family and components of the Src kinase family signal transduction pathways for the treatment of HBV infection and related disorders and diseases, such as HCC. The invention further relates to pharmaceutical compositions for the treatment of HBV infection targeted to HBx and its essential activities required to sustain HBV replication. The invention is based, in part, on the Applicants' discovery that activation of Src kinase signaling cascades play a fundamental role in mammalian hepadnavirus replication. Applicants have demonstrated that HBx mediates activation of the Src family of kinases and that this activation is a critical function provided by HBx for mammalian hepadnavirus replication.

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Abstract Paragraph - ABTX (1):

The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target HBx mediated activation of Src kinase, members of the Src tyrosine kinase family and components of the Src kinase family signal transduction pathways for the treatment of HBV infection and related disorders and diseases, such as HCC. The invention further relates to pharmaceutical compositions for the treatment of HBV infection targeted to HBx and its essential activities required to sustain HBV replication. The invention is based, in part, on the Applicants' discovery that activation of Src kinase signaling cascades play a fundamental role in mammalian hepadnavirus replication. Applicants have demonstrated that HBx mediates activation of the Src family of kinases and that this activation is a critical function provided by HBx for mammalian hepadnavirus replication.

Title - TTL (1):
Inhibition of the SRC kinase family pathway as a method of treating HBV infection and hepatocellular carcinoma

Summary of Invention Paragraph - BSTX (2):
[0001] The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target Src family kinases and components of the Src kinase family signal transduction pathways, including HBx activation of Src kinase family signal transduction pathways for the treatment and prevention of hepatitis B virus (HBV) infection and hepatocellular carcinoma (HCC). The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target cytosolic calcium release or calcium-dependent tyrosine kinase, Pyk2, which is the calcium entry point for activation of Src Kinases for the treatment and prevention of HBV infection and hepatocellular carcinoma. The invention also relates to screening assays to identify potential antiviral agents which target HBx-mediated activation of calcium-dependent tyrosine kinases and Src kinase signaling cascades for the treatment of HBV.

Summary of Invention Paragraph - BSTX (17):
[0014] The present invention relates to the treatment and revention of HBV infection by targeting activation of the Src family of kinases. The present invention further relates to the treatment and prevention of HBV infection by targeting activation of cytosolic calcium release and Pyk2-Src signal transduction. The present invention also relates to compounds which inhibit HBx-mediated activation of the Pyk2 tyrosine kinase and Src family of kinases as well as the downstream components of the Pyk2-Src kinase signaling cascade for the treatment of HBV infection.

Summary of Invention Paragraph - BSTX (19):
[0016] The Applicants have demonstrated that HBx mediated activation of Pyk2-Src kinase signaling cascade is an effective target for HBV anti-viral agents since activation of this pathway is essential for HBV replication. The Applicants have further demonstrated that HBx acts through calcium channels or their regulatory components to sustain HBV replication. Therefore, targeting HBx for the treatment of HBV should result in a highly specific and efficacious method of blocking HBV replication. The Pyk2-Src family of kinases, although host cell gene products, are only activated in proliferating or differentiating cells, and in cells infected by many DNA and tumor viruses. Therefore, targeting the Pyk2-Src kinase transduction pathway for the treatment of HBV infection should result in a therapeutic with a high degree of efficacy and sufficient specificity with side effects no more toxic than chemotherapeutics currently used to treat cancer.

Summary of Invention Paragraph - BSTX (20):
[0017] The present invention encompasses a variety of techniques and compounds to target the activities of HBx essential for HBV replication. In particular, these include, but are not limited to HBx-mediated activation of the Src kinase family signal transduction pathways for the treatment and prevention of HBV infection. The present invention encompasses the use of known inhibitors of Pyk2 tyrosine kinase signal transduction, in addition to inhibitors of calcium channels and their regulatory components, to treat HBV infection. The invention encompasses the use of known Src inhibitors to treat HBV infection. Examples of such specific inhibitors include, but not limited to: Pyk2 specific tyrosine kinase inhibitors, Src specific tyrosine kinase inhibitors, such as CsK, tyrphostin-derived inhibitors, derivatives of benzylidenemalonitrile, pyrazolopyrimidine PP1, and microbial agents, such as angelmicin B; and competitive inhibitors, such as small phosphotyrosine

containing ligands. The invention also encompasses the use of known HBx inhibitors for the treatment of HBV, including, but not limited to, antisense RNAs directed to HBx. The present invention also relates to the use of inhibitors of downstream effectors of Src kinases, including but not limited to, cytoplasmic factors, such as Ras, Raf, focal adhesion kinase (FAK) and MAPK, and nuclear factors, such as Myc and cyclin-dependent kinases.

Summary of Invention Paragraph - BSTX (21):
[0018] In another embodiment of the present invention gene therapy approaches, including dominant-negative mutants, antisense molecules and SELEX RNAs targeted to block Src kinase or HBx gene expression, may be used as a method to treat and prevent HBV infection and HCC. In yet another embodiment of the invention, upstream and downstream components and effectors of the Src kinase family signaling cascade may be targeted by gene therapy approaches to inhibit HBV infection.

Summary of Invention Paragraph - BSTX (22):
[0019] The present invention further relates to screening assays to identify compounds which inhibit HBx-mediated activation of the Src kinase signaling pathway and may be used to treat HBV infection and diseases and disorders associated with HBV infection. The present invention also relates to screening assays to identify compounds which inhibit HBx activation of Pyk2 tyrosine kinase and their regulatory components and may be used to treat HBV infection and diseases and disorders associated with HBV infection.

Summary of Invention Paragraph - BSTX (23):
[0020] The invention is illustrated by way of working examples which demonstrate that HBx mediates activation of a Pyk2-Src kinase signaling cascade and that activation of this signaling cascade is an essential function of HBx required to sustain HBV replication. The working examples of the present invention further demonstrate the ability of inhibitors of the Src kinase signaling cascade to inhibit HBV replication.

Summary of Invention Paragraph - BSTX (26):
[0023] As used herein, the term "target protein" refers to those proteins which correspond to Src kinase or members of the Src kinase family or components of the Src kinase signaling pathway or proteins encoded by the HBV genome, including HBx.

Brief Description of Drawings Paragraph - DRTX (16):
[0045] FIG. 6. Woodchuck Hepatitis B Virus (WHV) HBx protein (WHx) activates a Src-Ras signaling cascade during WHV replication in cultured cells. Chang cells were co-transfected with 20 .mu.g pcWHV or wtWHV with 8 .mu.g of either dominant-negative Ras, kinase inactive (dominant-negative) Src, or Csk plasmids. Eighteen hours post-transfection, cells were serum-starved in 0.5% CS for 20 hours, MAP kinase (ERK-2) was immunoprecipitated from equal amounts of cell lysates and pellets were subjected to an in vitro MBP kinase assay.

Brief Description of Drawings Paragraph - DRTX (17):
[0046] FIG. 7. WHx requires activation of Src family kinase for WHV replication. Chang cells were co-transfected with 20 .mu.g PCWHV, wtWHV, wtWHV and RasDN (dominant-negative) (10 .mu.g), or wtWHV and Csk (10 .mu.g). Three days post-transfection viral core-associated DNA was isolated, purified, and subjected to Southern blot analysis using a full-length .sup.32P-labeled WHV genomic probe.

Detail Description Paragraph - DETX (2):

[0052] The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target HBx-mediated activation of calcium-dependent tyrosine kinase, PhK2, HBx-mediated activation of Src kinase, members of the Src tyrosine kinase family and components of the Src kinase family signal transduction pathways for the treatment of HBV infection and related disorders and diseases, such as HCC. The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target cytosolic calcium release, regulation of calcium channels and thus, inhibit HBx-mediated activation of calcium-dependent tyrosine kinase Pyk2. The invention further relates to pharmaceutical compositions for the treatment of HBV-infection targeted to HBx and its essential activities required to sustain HBV replication.

Detail Description Paragraph - DETX (3):

[0053] The invention is based, in part, on the Applicants' discovery that activation of Pyk2-Src kinase signaling cascades plays a fundamental role in mammalian hepadnavirus replication. Applicants have demonstrated that HBx mediates activation of Pyk2, FAK, Src and MAPK signalling all occur in a calcium-dependent manner and that this activation is a critical function provided by HBx for mammalian hepadnavirus replication.

Detail Description Paragraph - DETX (9):

[0059] 5.1 The Role of HBx Mediated Calcium Dependent Activation of Pyk2 Tyrosine Kinase and SRC Kinase Activation in HBV-infection and its use as a Target for Intervention

Detail Description Paragraph - DETX (10):

[0060] The present invention is based, in part, on the Applicants' surprising discovery that (1) HBx requires activation of cytosolic calcium release, and calcium channels or their regulatory components to sustain HBV replication; (2) HBx acts as an intracellular cytoplasmic activator of the Pyk2 tyrosine Kinase, (3) HBx acts as an intracellular, cytoplasmic activator of the Src family of nonreceptor tyrosine kinases; (4) HBx stimulates tyrosine kinase activity of the Src family kinase members, including c-Src and c-Fyn; and (5) inhibition of Src activity by the expression of a Src inhibitor, e.g., the Csk protein, results in the dramatic inhibition of HBV replication. This discovery is exemplified in the in Sections 6, 7, 8 and 9 infra, which demonstrate that activation of Src kinase and the Src kinase signaling cascade is required to sustain HBV replication, and that inhibition of Src kinase dramatically inhibits HBV replication.

Detail Description Paragraph - DETX (17):

[0067] Applicants have further demonstrated that the expression a Src inhibitors, i.e., Csk protein, or dominant-negative Src or Fyn proteins resulted in the inhibition of HBx activation of downstream components of Src kinase signaling cascade. Applicants have also shown that the expression of Src dominant-negative mutants, such as Csk, inhibited the ability of HBx to stimulate activities of the nuclear factor, Myc, including stimulation of cell cycle progression by blocking HBx activation of Src kinase signaling pathways. These findings clearly establish that activation of a Src kinase signaling cascade by HBx has a critical role in the hepadnaviral life cycle.

Detail Description Paragraph - DETX (18):

[0068] HBx mediated activation of Src is required for HBV replication as demonstrated by way of example (Section 9 infra). The Applicants' work demonstrates that an essential component of the requirement of HBx viral replication in cultured cells is its ability to activate Src signaling cascades. HBx activation of a Src signaling cascade plays a critical role in transcriptional upregulation of the viral mRNAs. Inhibition of Src activity by

the expression of a Src inhibitor, e.g., the CsK protein, results in the dramatic inhibition of HBV replication. These results illustrate that activation of Src family kinases has an essential role during HBV replicative life cycles.

Detail Description Paragraph - DETX (19):
[0069] The Applicants' discovery has implicated several targets for effective HBV anti-viral agents. HBV therapies that target the viral gene product HBx should result in a high degree of specificity and efficacy. HBV therapies that target the host cell gene products, including, but not limited to, regulatory components of cytosolic calcium release, including mitochondrial and ER calcium channels, Pyk2 kinases, the Src family of kinases, should likewise demonstrate specificity and efficacy. Although host cell gene products, Pyk2 kinases and the Src family of kinases are active in proliferating cells, such as cancer cells, or in virally infected cells. Therefore, targeting the Src family of kinases for the treatment of HBV infection should result in a high degree of efficacy, and sufficient specificity with side effects no more toxic than chemotherapeutics currently used to treat cancer.

Detail Description Paragraph - DETX (20):
[0070] 5.2 Treatment of HBV-infection using Inhibitors of HBx Mediated Src Activation

Detail Description Paragraph - DETX (21):
[0071] The present invention encompasses a variety of therapeutic protocols, methods and compounds to target HBx-mediated activation of cytosolic calcium release including mitochondrial and ER calcium channels, the Pyk2 signalling cascade, and the Src kinase signaling cascade for the treatment of HBV. The present invention encompasses all of the compounds described in the subsections below to target HBx-mediated activation of cytosolic calcium release including mitochondrial and ER calcium channels as targets, the Pyk2 signalling cascade, and the Src kinase signaling cascade with the proviso that they are not known in the art to be used to treat HBV infection, including, for example, interferon .alpha., interferon .delta., interleukin-1, interleukin-2, immune-active peptides, such as thymosin-alpha, nucleoside analogs, such as vidarabine, fialuridine, lamivuridine, famciclovir, ribavarin, and corticosteroids, such as prednisone and azathioprine.

Detail Description Paragraph - DETX (29):
[0079] A variety of techniques and compositions may be utilized to target Src kinase to inhibit its activity or to inhibit HBx mediated activation of components of the Src kinase mediated signaling cascade, thereby inhibiting HBV replication. Such techniques and compositions may include, but are not limited to, gene therapy approaches, drugs, small organic molecules identified to inhibit Src kinase, Ras, Raf, MAPK kinase, MAPK, c-Myc, cyclin-dependent kinases and/or other downstream effectors of the Src kinase signaling cascade.

Detail Description Paragraph - DETX (30):
[0080] In particular, compounds which may be used in accordance with the present invention to specifically target activation of Src kinase are binding proteins and competing ligands that prevent the intramolecular interaction between the carboxyterminal phosphorylated tyrosine residue and the SH2 domain located in the amino-terminal half of the molecule and the immediately adjacent SH3 domain (Lin et al., 1993, Oncogene 8:1119-1126). In particular, compounds which may also be used in accordance with the present invention include tyrosine kinase inhibitors which block the activity the Src kinase signaling cascade and therefore would block HBV replication. Examples of such tyrosine kinase inhibitors include, but are not limited to, tyrphostin-derived

inhibitors, which are derivatives of benzylidenemalonitrile, have been shown to have strong inhibitory activity of Src (Ramdas et al., 1995, Archives of Biochemistry and Biophysics 323:237-242), pyrazolopyrimidine PP1 (4-amino-5-(4methylphenyl)-7-(t-butyl) pyrazolo [3,4-d] pyrimidine, a selective inhibitor of the Src family of kinases (Hanke et al., 1996, J. Biol. Chem. 271:695-791) and derivatives thereof. Other examples include microbial agents, such as angelmicin B, a specific inhibitor of Src tyrosine kinase activity, and derivatives thereof (Yokoyama et al., 1996, Leukemia Research 20:491-497), which may also be used to inhibit HBV replication.

Detail Description Paragraph - DETX (31):

[0081] In another embodiment of the present invention, small peptides which compete with larger phosphotyrosine peptides for binding to the Src kinase protein may be used to inhibit the PkY2-Src kinase signaling cascade, in particular small phosphotyrosine containing peptide ligands, 5 to 6 amino acids, which are able to compete with larger phosphotyrosine-containing peptides and protein ligands for binding to SH2 domains, thereby inhibiting the Pyk2-Src kinase signaling cascade and blocking replication of HBV. Another embodiment of the present invention includes small peptides which correspond to catalytic or enzymatic domains of Pyk2 kinase, Src kinase and would compete with the respective kinase, inhibiting the activation of downstream components of the Pyk2-Src kinase signaling cascade. Another embodiment includes the use of larger polypeptides that inhibit Src kinase activity including, but not limited to, Csk (carboxyl-terminal Src kinase) which is a specific physiologic inhibitor of Src kinase. Further examples of larger polypeptides that inhibit Src kinase activity include, for example, Src dominant-negative mutants, i.e., Srck-(Barone et al., 1995, Nature 378:509-512) and Fyn dominant-negative mutants (Twamley-Stein et al., 1993, Proc. Natl. Acad. Sci. USA 90:7696-7700), also included are dominant-negative mutants of downstream effectors of the Src kinase signaling cascade, including Ras, Raf, MAPK kinase, MAPK dominant-negative mutants and Myc dominant-negative mutants (Sawyers et al., 1992, Cell 70:901-910).

Detail Description Paragraph - DETX (34):

[0084] In one embodiment of the invention, novel antiviral agents identified by the screening methods of the present invention are used in combination with known therapies to treat HBV infection, for example, IFN, interleukin-1, interleukin-2, immune-active peptides, nucleoside analogs and corticosteriods. The antiviral agents identified by the screening methods of the present invention may also be used in combination with exogenous or endogenous agents which induce IFN expression. In yet another embodiment, inhibitors of Src kinase are used in combination with agents which induce an anti-HBV immune response in order to target two different molecules required in the viral life cycle.

Detail Description Paragraph - DETX (53):

[0103] In yet another specific embodiment, attenuated viruses, such as hepadnaviruses, which have the same tropism as HBV, may be engineered and used for gene therapy in accordance with the present invention. Hepadnaviruses are particularly attractive for use in gene therapy in accordance with the present invention as these viruses will deliver the therapeutic exactly to those cells infected with HBV. Hepadnaviral vectors would be particularly effective for the delivery of nucleic acids targeting components of the Src kinase signaling cascade, thereby avoiding unnecessarily knocking out expression of host genes.

Detail Description Paragraph - DETX (95):

[0145] At least two different assay systems, described in the subsections below, can be designed and used to identify compounds or compositions that modulate HBx-mediated activation of Src kinase signaling cascades and thereby

inhibit HBV replication.

Detail Description Paragraph - DETX (96):
[0146] The systems described below may be formulated into kits. To this end, cells expressing HBx and components of the Src kinase signaling cascade, or cells expressing components of the Src kinase signaling cascade which are capable of sustaining HBV replication, or cell lysates thereof can be packaged in a variety of containers, e.g., vials, tubes, microtitre well plates, bottles, and the like. Other reagents can be included in separate containers and provided with the kit; e.g., positive control samples, negative control samples, buffers, cell culture media, etc.

Detail Description Paragraph - DETX (100):
[0150] Alternately, cell lines which co-express HBx and Src kinase and components of the Src kinase signaling cascade may be genetically engineered to assay for inhibitors of HBx activation of Src. This can be engineered in cell in the absence of HBV replication or in cell lines which support the HBV life cycle as a means of (1) identifying additional factors required to support the HBV life cycle, and (2) as a system to screen test compounds, for their ability to interfere with HBx activation and/or interaction with the Src kinase, and (3) as a system to screen test compounds for their ability to inhibit Src kinase activity and therefore inhibit HBV replication.

Detail Description Paragraph - DETX (109):
[0159] Alternatively, activation of Src kinase signaling pathways mediated by HBx may be measured by the secretion of mature HBV viral particles into the medium of growing Chang cells. For example, Chang liver cells may be stably transformed with an HBV or WHV pregenome, or with a head-to-tail dimer of either HBV or WHV genomes. The integrated virus will produce and secrete HBV/WHV particles into the medium. As demonstrated by the Applicants, the secretion of viral particles is strongly enhanced by HBx protein activation of Src kinases. If the test compound is effective in inhibiting HBx activation of Src, it will result in reduced secretion of HBV/WHV particles into the medium. The level of particle secreted into the medium can be assayed using commercial ELISA kits to detect the presence of HBV/WHV HBcAg and HBsAg.

Detail Description Paragraph - DETX (115):
[0165] In preferred embodiment of the invention, Src kinase is expressed alone in transgenic Src knock-out mice and a HBV pseudovirus is used to infect the animals. For example, a HBV pseudovirus which contains the HBV virus and an envelope protein from a virus with a natural tropism for murine cells, such as the murine leukemia virus (MLV), is used to bypass internalization of the HBV virus by the murine cells. These murine cells can then support the life cycle of the internalized HBV virus, because they express human Src kinase.

Detail Description Paragraph - DETX (117):
[0167] In yet another embodiment of the animal model screens of the present invention, the effect of test compounds to inhibit HBV replication may be measured indirectly. For example, transgenic mice may be engineered which express (1) the HBx gene product under the control of an inducible promoter, and (2) readout vector which is responsive to Src activation. The readout vector may comprise a reporter gene under the control of a Myc promoter. Such reporter constructs are described in Section 5.5.1 infra. In this assay system, expression of the HBx gene product is induced and the test compound is administered to the mice. The ability of the test compound to inhibit HBx mediated activation of Src kinase and HBV replication is assayed by measuring the reporter gene. Such reporter genes may include but are not limited to chloramphenicol acetyltransferase (CAT), luciferase, GUS, growth hormone, or placental alkaline phosphatase (SEAP). Following exposure of the animal to the

test compound, the level of reporter gene expression may be quantitated from the blood or tissue sample to determine the test compound's ability to regulate receptor activity. Alkaline phosphatase assays are particularly useful in the practice of the invention as the enzyme is secreted from the cell. Therefore, tissue culture supernatant may be assayed for secreted alkaline phosphatase. In addition, alkaline phosphatase activity may be measured by calorimetric, bioluminescent or chemilumenscent assays such as those described in Bronstein, I. et al. (1994, Biotechniques 17: 172-177). Such assays provide a simple, sensitive easily detection system for pharmaceutical screening.

Detail Description Paragraph - DETX (173):
WHV Requires Src Family Kinases for in Vitro Replication

Detail Description Paragraph - DETX (178):
[0220] Chang cells were co-transfected with plasmids encoding either wtWHV, PCWHV, wtWHV and RasDN, or wtWHV and Csk, intracellular core-associated viral DNA was isolated 3 days post-transfection, and purified viral DNA analyzed by Southern blot hybridization as described above (FIG. 7). Consistent with the previous data, accumulation of viral DNA replicative intermediates was strongly enhanced in cells expressing wtWHV, as compared to cells expressing PCWHV. Co-expression of RasDN protein with wtWHV had no detectable effect on viral replication, and viral DNA was synthesized at near wild-type levels. Expression of the RasDN protein impaired WHV activation of MAP kinase under these same experimental conditions (FIG. 7), indicating that the RasDN protein was able to function during WHV replication. However, these results illustrate the effects of one particular inhibitor of Ras and do not provide an explanation of the mechanism by which activation of Src kinase supports HBV replication. In sharp contrast, co-expression of Csk with wtWHV completely abolished the ability of wtWHV to replicate to high levels. These results demonstrated that an essential component of the requirement of HBx during in vitro viral replication in Chang cells is its ability to activate Src signaling cascades, and that activation of Src family kinases has a critical role during the viral replicative life cycle.

Detail Description Paragraph - DETX (180):
[0222] To assess whether HBx activation of a Src signaling cascade plays an essential role in transcriptional upregulation of the viral mRNAs, Chang cells were co-transfected with wtWHV and either RasDN or Csk plasmids, and the RNA visualized by Northern analysis (FIG. 7, lanes 2 and 3). Co-expression of RasDN with wtWHV only slightly reduced the amount of the RNA species, while co-expression of wtWHV with Csk reduced the RNA level .about.3-5 fold, to the level also observed by expression of pCWHV. To ensure that the decrease in synthesis of WHV RNA by Csk was not the unforseen consequence of Csk inhibition of the CMV promoter (which drives synthesis of the WHV pregenomic RNA), control experiments assessing the effect of Csk on a CMV-.beta.gal reporter were carried out. In comparison with expression of CMV-.beta.gal alone, co-expression of Csk with CMV-.beta.gal did not significantly alter expression of the .beta.gal protein as measured by its .beta.-galactosidase activity (Sambrook et al, 1989, supra). This control experiment indicates that expression of Csk does not generally inhibit transcription of the CMV promoter, and rules out a non-specific effect of Csk on viral transcription. Therefore, these data suggest that the HBx protein moderately increases the abundance of all the viral transcripts through activation of the Src family of kinases. However, the WHx-induced increase in mRNA abundance (.about.3-5 fold) is much less then the HBx-induced increase in viral DNA synthesis (.about.20-30 fold). Therefore, transcriptional transactivation by HBx does not appear to fully account for the augmentation of viral replication by the HBx protein. The stimulation of Src signaling cascades by HBx must therefore promote WHV replication independent of the effect of WHx on viral transcription. These

results illustrate that HBx activates a Src-Ras signaling cascade during viral replication in vitro which is essential for the host cell to sustain HBV replication.

Detail Description Paragraph - DETX (182):
WHV Requires Src Family of Kinases for in Vivo Replication

Detail Description Paragraph - DETX (183):
[0223] The requirement for activation of Src kinase family members in replication of WHV can be determined in woodchuck infected livers in the following manner. A 2-5 year old woodchuck is experimentally infected using a pooled serum from previous chronic carrier woodchucks. After 2 years of chronic infection, determined by WHsAg ELISA, the infected liver is surgically removed, the liver is perfused as described (Jacob et al., 1994, Exp. Cell Res. 212:42-48), hepatocytes are dispersed by collagenase treatment and plated onto collagen coated dishes in L15 medium supplemented with 5% fetal calf serum, hydrocortisone and insulin. To introduce an inhibitor of Src kinases into primary hepatocytes, the Csk gene is cloned into the left-end of a replication-defective adenovirus vector under the control of the CMV promoter, as described (Doria et al., 1995, EMBO J. 14:4747-4757). Adenovirus vectors infect primary cells and express trans-genes efficiently, whereas it is not possible to transfect such cells at a high rate. Within several days of plating, cells are infected with the Csk-adenovirus vector, medium is replaced with L15 medium lacking insulin and containing reduced serum (between 0.5-2%). Cells are then harvested at 2 and 4 days after introduction of the vector. The medium can be assayed for levels of secreted WHV by ELISA for WHcAg and WHsAg. The level of virus replication can be assayed as described for Chang cells.

Detail Description Paragraph - DETX (194):
[0232] In summary, a major finding of this work is that HBx acts on cytosolic stored calcium to stimulate Pyk2-Src kinase signal transduction pathways that activate HBV reverse transcription and DNA replication, and in some instances, to function as a moderate transcriptional activator. Three lines of evidence indicate that HBx stimulation of HBV reverse transcription/DNA replication involves alteration of cytosolic calcium and activation of Pyk2-Src kinase signal transduction. First, activation of Pyk2, which is critical for stimulation of HBV DNA replication in tissue culture, is typically mediated by increased levels of cytosolic calcium. Chelation of cytosolic calcium with BAPTA-AM blocked HBx activation of Pyk2 and HBV DNA replication. Second, inhibition of mitochondrial and possibly ER calcium channels with CsA blocked HBx activation of HBV DNA replication. Third, ionophoric agents that increase the level of cytoplasmic calcium functionally replace HBx in viral DNA replication. Thus, HBx acts on stored cytosolic calcium as a fundamental activity for HBV replication.

PGPUB-DOCUMENT-NUMBER: 20010044521

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20010044521 A1

TITLE: Universal procedure for refolding recombinant proteins

PUBLICATION-DATE: November 22, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
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| Lin, Xinli | Edmond | OK | US | |

APPL-NO: 09/ 752878

DATE FILED: December 28, 2000

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60177836 20000125 US

non-provisional-of-provisional 60178368 20000127 US

non-provisional-of-provisional 60210292 20000608 US

non-provisional-of-provisional 60210306 20000608 US

US-CL-CURRENT: 530/350, 435/69.1

ABSTRACT:

A universal folding method that has been demonstrated to be effective in refolding a variety of very different proteins expressed in bacteria as inclusion bodies has been developed. Representative proteins that can be dissolved and refolded in biologically active form, with the native structure, are shown in Table I. The method has two key steps to unfold and then refold the proteins expressed in the inclusion bodies. The first step is to raise the pH of the protein solution in the presence of denaturing agents to pH greater than 9, preferably 10. The protein solution may be maintained at the elevated pH for a period of up to about 24 hours, or the pH immediately decreased slowly, in increments of about 0.2 pH units/24 hours, until the solution reaches a pH of about 8.0, or both steps used. In the preferred embodiment, purified inclusion bodies are dissolved in 8 M urea, 0.1 M Tris, 1 mM glycine, 1 mM EDTA, 10 mM beta-mercaptoethanol, 10 mM dithiothreitol (DTT), 1 mM redued glutathion (GSH), 0.1 mM oxidized glutathion (GSSG), pH 10. The absorbance at 280 nm (OD280) of the protein solution is 5.0. This solution is rapidly diluted into 20 volumes of 20 mM Tris base. The resulting solutin is adjusted to pH 9.0 with 1 M HCl and is kept at 4.degree. C. for 24 hr. The pH is adjusted to pH 8.8 and the solution is kept at 4.degree. C. for another 24 hrs. This process is repeated until the pH is adjusted to 8.0. After 24 hr at pH 8.0, the refolded proteins can be concentrated by ultrafiltration and applied to a gel filtration column for purification.

---------- KWIC ---------

Summary of Invention - Table CWU - BSTL (1):

1TABLE I Expression, refolding, and purification of different proteins from E. coli Purifi- Name From Organism Refold cation Ref. Pepsinogen Full-length Porcine Yes Yes Lin, et al, 1989 Pepsinogen N and C Porcine Yes Yes Lin, et al, 1992 Domain Lin, et al, 1993 Rhizopus- full-length Fungus Yes Yes Chen, et al, 1991 Pepsinogen Lin, et al, 1992 Thermopsin full-length Archae No No None Thermopsin fusion Archae Yes Partial Lin, Liu, Tang, 92 Cathepsin D full-length human No No None low yield Pregnancy full-length Bovine No No None Specific Ant Ovine HIV protease full-length HIV Yes Yes Lin, et al, 1995 Ermolief, et al, 97 SAP full-length Yeast Yes Yes Lin, et al, 1993 Koelsch, et al, 98 Streptokinase full-length bacteria Yes Yes Wang, et al, 1998 Plasminogen cat-domain human Yes Yes Wang, et al, 2000 Cadosin A full-length plant Yes Yes Faro, et al, 1999 Napsin 1 full-length human No No Koelsch, et al, 00 Memapsin 2 full-length human Yes Yes Lin, et al, 2000 Memapsin 1 full-length human Yes Yes PreS partial HBV Yes Yes unc-76 full-length C. elegans Yes Yes odc-1 full-length C. elegans Yes Yes ceh-10 full-length C. elegans Yes Yes ppp-1 full-length C. elegans No No

US-PAT-NO: 6884804

DOCUMENT-IDENTIFIER: US 6884804 B2

TITLE: Inhibitors of Src and other protein kinases

DATE-ISSUED: April 26, 2005

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
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APPL-NO: 10/ 146984

DATE FILED: May 16, 2002

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application 60/291,340 filed May 16, 2001, the contents of which are incorporated herein by reference.

US-CL-CURRENT: 514/275, 514/227.8 , 514/235.8 , 514/236.8 , 514/241 , 544/122 , 544/180 , 544/212 , 544/219 , 544/238 , 544/298 , 544/331 , 544/60

ABSTRACT:

The present invention provides compounds of formula I: ##STR1##

wherein A is N or CR, and G, R.sup.1, R.sup.2 and R.sup.3 are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of Src mammalian protein kinase involved in cell proliferation, cell death and response to extracellular stimuli. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

26 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (9):

Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J., 18, 5019, (1999) and Klein et al., Mol.Cell. Biol., 17, 6427 (1997).

US-PAT-NO: 6846928

DOCUMENT-IDENTIFIER: US 6846928 B2

TITLE: Compositions useful as inhibitors of protein kinases

DATE-ISSUED: January 25, 2005

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
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| Bebbington; David | Newbury | N/A | N/A | GB |
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| Everitt; Simon | Beaconsfield | N/A | N/A | GB |
| Golec; Julian M. C. | Ashbury | N/A | N/A | GB |
| Kay; David | Purton | N/A | N/A | GB |
| Knegtel; Ronald | Abingdon | N/A | N/A | GB |
| Miller; Andrew | Upton | N/A | N/A | GB |
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APPL-NO: 10/ 389707

DATE FILED: March 14, 2003

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Patent Application No. 60/364,842 filed Mar. 15, 2002 the entirety of which is incorporated herein by reference.

US-CL-CURRENT: 544/316

ABSTRACT:

The present invention relates to compounds useful as inhibitors of protein kinases. The invention also provides pharmaceutically acceptable compositions comprising said compounds and methods of using the compositions in the treatment of various disease, conditions, or disorders.

12 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (18):

Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J. 1999, 18, 5019, and Klein et al., Mol. Cell. Biol. 1997, 17, 6427.

US-PAT-NO: 6750009

DOCUMENT-IDENTIFIER: US 6750009 B2

TITLE: Multiple viral replicon culture systems

DATE-ISSUED: June 15, 2004

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
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APPL-NO: 10/ 060941

DATE FILED: January 29, 2002

US-CL-CURRENT: 435/5, 435/347 , 435/373 , 435/455

ABSTRACT:

Methods and compositions are provided for screening candidate antiviral agents using cells containing subgenomic viral replication systems such as replicons and minigenomes. The methods involve the simultaneous assay of more than one subgenomic viral replication system. Compositions useful for these methods are also provided.

23 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

---------- KWIC ---------

Detailed Description Paragraph Table - DETL (1):

TABLE 1 Viral replicons for antiviral screening Infec- tious Noncytopathic Family Virus (common names) clone replicon Togaviridae Sindbis yes yes Venezuela encephalitis virus yes possible Rubella yes possible Picornaviridae Poliovirus yes yes Coxsackirus yes possible Enterovirus yes possible Hepatitis A yes possible Flaviviridae Yellow fever yes yes Dengue fever yes possible West Nile virus possible Japanese Encephalitis virus yes yes Hepatitis C virus yes yes Tick-born encephalitis virus possible (TBE) Astroviridae Astrovirus yes possible Rhabdoviridae Rabies virus yes possible Orthomyxoviridae Influenza virus A yes possible Influenza virus B possible Paramyxoviridae Respiratory syncytial virus yes possible (RSV) Measles yes possible Mumps yes possible Filoviridae Ebola yes possible Marburg possible Bunyaviridae La Crosse virus possible California encephalitis virus yes possible Hantaan virus possible Crimean-Congo possible Rift Valley fever possible Arenaviridae Lassa fever possible Argentine Hemorrhagic fever possible Bolivian Hemorrhagic fever possible Reoviridae Colorado tick fever Hepadanviridae **Hepatitis B virus yes** yes Papillomaviridae Human papilloma virus yes yes Polyomaviridae JC virus yes possible BK virus yes possible Herpeviruses Herpes simplex virus type yes yes one (HSV-1) Herpes simplex

virus type yes possible two (HSV-2) Epstein-Barr virus (EBV) yes yes Human cytomegalovirus yes possible (HCMV) Varicella-zoster virus (VZV) yes possible Human herpes virus type poss- possible six (HHV6) ible Human herpes virus type possi- possible seven (HHV7) ible Human herpes virus type poss- possible eight (HHV8) ible Adenoviridae Human adenovirus yes possible Retrovirus Human immunodeficiency yes possible virus type one (HIV-1) Human immunodeficiency yes possible virus type two (HIV-2) Human t-cell leukemia virus yes possible type one (HTLV-1) Human t-cell leukemia virus yes possible type two (HTLV-2) Parvoviridae Human parvovirus yes possible Adeno-associated virus yes yes

US-PAT-NO: 6689778

DOCUMENT-IDENTIFIER: US 6689778 B2

TITLE: Inhibitors of Src and Lck protein kinases

DATE-ISSUED: February 10, 2004

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
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| Salituro; Francesco | Marlboro | MA | N/A | N/A |
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| Ledeboer; Mark | Acton | MA | N/A | N/A |


APPL-NO: 10/ 171895

DATE FILED: June 14, 2002

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Patent Application No. 60/302,969 filed Jul. 3, 2001, the contents of which are incorporated herein by reference.

US-CL-CURRENT: 514/235.8, 514/252.19 , 514/275 , 544/122 , 544/331

ABSTRACT:

The present invention provides compounds of formula I: ##STR1##

or a pharmaceutically acceptable derivative thereof, wherein A--B is N--O or O--N and G, R.sup.1, R.sup.2, R.sup.3, and R.sup.4 are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of Src and Lck kinase. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

17 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (9):

Src also plays a role in the replication of hepatitis B virus. The virally encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J., 18, 5019, (1999) and Klein et al., Mol.Cell. Biol., 17, 6427 (1997).

US-PAT-NO: 6642227

DOCUMENT-IDENTIFIER: US 6642227 B2

TITLE: Inhibitors of c-Jun N-terminal kinases (JNK) and other protein kinases

DATE-ISSUED: November 4, 2003


INVENTOR-INFORMATION:

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| Wang; Jian | Boston | MA | N/A | N/A |
| Ledeboer; Mark | Acton | MA | N/A | N/A |
| Harrington; Edmund | South Boston | MA | N/A | N/A |
| Gao; Huai | Natick | MA | N/A | N/A |



APPL-NO: 10/ 121035

DATE FILED: April 10, 2002


PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims benefit of priority to U.S. provisional applications 60/283,621 filed Apr. 13, 2001, 60/329,440 filed Oct. 15, 2001 and 60/292,974 filed May 23, 2001.


US-CL-CURRENT: 514/227.8, 514/235.8 , 514/252.19 , 514/275 , 544/122 , 544/331 , 544/60



ABSTRACT:

The present invention provides compounds of formula I: ##STR1##

or a pharmaceutically acceptable derivative o thereof, wherein A, B, R.sup.a, R.sup.1, R.sup.2, R.sup.3, and R.sup.4 are as described in the specification. These compounds are inhibitors of protein kinase, particularly inhibitors of JNK, a mammalian protein kinase involved cell proliferation, cell death and response to extracellular stimuli; Lck and Src kinase. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.


31 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC --------

Brief Summary Text - BSTX (19):
Src also plays a role in the replication of hepatitis B virus. The virally

encoded transcription factor HBx activates Src in a step required for propagation of the virus. Klein et al., EMBO J., 18, 5019, (1999) and Klein et al., Mol. Cell. Biol., 17, 6427 (1997).

US-PAT-NO: 6589734

DOCUMENT-IDENTIFIER: US 6589734 B1

TITLE: Detection of HIV

DATE-ISSUED: July 8, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
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| McDonough; Sherrol H. | San Diego | CA | N/A | N/A |


APPL-NO: 09/ 168947

DATE FILED: October 8, 1998

PARENT-CASE:

This application is a continuation-in-part of application Ser. No. 08/469,067, filed Jun. 6, 1995, now U.S. Pat. No. 5,824,518, which is a continuation of application Ser. No. 07/550,837, filed Jul. 10, 1990, now U.S. Pat. No. 5,480,784, which is a continuation-in-part of application Ser. No. 07/379,501, filed Jul. 11, 1989, now abandoned, with application Ser. Nos. 08/469,067 and 07/550,837 being hereby incorporated by reference herein in their entirety (including the drawings).

US-CL-CURRENT: 435/6, 435/91.2 , 435/91.21 , 536/23.1 , 536/24.32

ABSTRACT:

The present invention related to oligonucleotides for use in amplifying and detecting HIV nucleic acid in a sample.

110 Claims, 28 Drawing figures

Exemplary Claim Number: 1,13,25

Number of Drawing Sheets: 26

---------- KWIC ---------

Detailed Description Paragraph Table - DETL (5):
TABLE 5 Preliminary Procedure II Reaction Kinetics. Reaction 1 2 3 4 5 6 7 Target (10 amol) No Yes Yes Yes Yes Yes Yes T7pro(+) Yes No Yes No No Yes Yes T7pro(-) Yes No No Yes No No Yes HBV(-)Pr Yes No No No Yes Yes No Time (minutes) Minus Product (RLU's) 0 619 638 635 703 592 619 656 30 613 635 613 755 626 844 1133 60 635 649 856 894 635 2146 6008 90 593 619 619 925 624 6226 23484 120 621 606 627 946 639 12573 43939 180 678 635 714 930 627 21719 78682 Time (minutes) Plus Product (RLU's) 0 624 646 1661 710 621 636 962 30 637 601 602 629 655 803 758 60 639 706 800 679 664 226 2895 90 638 683 956 633 687 7786 8085 120 643 670 884 647 632 18160 18241 180 683 617 968 758 712 34412 41165

Detailed Description Paragraph Table - DETL (6):

TABLE 6 Preliminary Procedure II System. Reaction 1 2 3 4 5 6 7 M13L(-) No No Yes Yes Yes Yes Yes T7pro(+) Yes Yes No Yes No Yes Yes T7pro(-) Yes No No No Yes Yes No HBV(-)Pr No Yes No No No No Yes Probe Relative Light Units (RLU's) Probe(+) 862 744 762 1089 2577 96221 30501 Probe(-) 473 420 483 3038 1080 15171 14863

US-PAT-NO: 6583268

DOCUMENT-IDENTIFIER: US 6583268 B2

TITLE: Universal procedure for refolding recombinant proteins

DATE-ISSUED: June 24, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
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| Lin; Xinli | Edmond | OK | N/A | N/A |

APPL-NO: 09/ 752878

DATE FILED: December 28, 2000

PARENT-CASE:

This application claims priority to U.S. S. No. 60/177,836 filed Jan. 25, 2000 by Lin, et al., U.S. S. No. 60/178,368 filed Jan. 27, 2000 by Lin, et al., and U.S. S. No. 60/210,292 filed Jun. 8, 2000 by Hong, et al., and to U.S. S. No. 60/210,306 filed Jun. 8, 2000 by Lin.

US-CL-CURRENT: 530/350, 435/69.1 , 530/412 , 530/417

ABSTRACT:

A universal folding method that has been demonstrated to be effective in refolding a variety of very different proteins expressed in bacteria as inclusion bodies has been developed. Representative proteins that can be dissolved and refolded in biologically active form, with the native structure, are shown in Table I. The method has two key steps to unfold and then refold the proteins expressed in the inclusion bodies. The first step is to raise the pH of the protein solution in the presence of denaturing agents to pH greater than 9, preferably 10. The protein solution may be maintained at the elevated pH for a period of up to about 24 hours, or the pH immediately decreased slowly, in increments of about 0.2 pH units/24 hours, until the solution reaches a pH of about 8.0, or both steps used. In the preferred embodiment, purified inclusion bodies are dissolved in 8 M urea, 0.1 M Tris, 1 mM glycine, 1 mM EDTA, 10 mM beta-mercaptoethanol, 10 mM dithiothreitol (DTT), 1 mM redued glutathion (GSH), 0.1 mM oxidized glutathion (GSSG), pH 10. The absorbance at 280 nm (OD280) of the protein solution is 5.0. This solution is rapidly diluted into 20 volumes of 20 mM Tris base. The resulting solutin is adjusted to pH 9.0 with 1 M HCl and is kept at 4.degree. C. for 24 hr. The pH is adjusted to pH 8.8 and the solution is kept at 4.degree. C. for another 24 hrs. This process is repeated until the pH is adjusted to 8.0. After 24 hr at pH 8.0, the refolded proteins can be concentrated by ultrafiltration and applied to a gel filtration column for purification.

15 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Paragraph Table - BSTL (1):

TABLE I Expression, refolding, and purification of different proteins from E. coli Purifi- Name From Organism Refold cation Ref. Pepsinogen Full-length Porcine Yes Yes Lin, et al, 1989 Pepsinogen N and C Porcine Yes Yes Lin, et al, 1992 Domain Lin, et al, 1993 Rhizopus- full-length Fungus Yes Yes Chen, et al, 1991 Pepsinogen Lin, et al, 1992 Thermopsin full-length Archae No No None Thermopsin fusion Archae Yes Partial Lin, Liu, Tang, 92 Cathepsin D full-length human No No None low yield Pregnancy full-length Bovine No No None Specific Ant Ovine HIV protease full-length HIV Yes Yes Lin, et al, 1995 Ermolief, et al, 97 SAP full-length Yeast Yes Yes Lin, et al, 1993 Koelsch, et al, 98 Streptokinase full-length bacteria Yes Yes Wang, et al, 1998 Plasminogen cat-domain human Yes Yes Wang, et al, 2000 Cadosin A full-length plant Yes Yes Faro, et al, 1999 Napsin 1 full-length human No No Koelsch, et al, 00 Memapsin 2 full-length human Yes Yes Lin, et al, 2000 Memapsin 1 full-length human Yes Yes PreS partial HBV Yes Yes unc-76 full-length C. elegans Yes Yes odc-1 full-length C. elegans Yes Yes ceh-10 full-length C. elegans Yes Yes ppp-1 full-length C. elegans No No

US-PAT-NO: 6503914

DOCUMENT-IDENTIFIER: US 6503914 B1
**See image for Certificate of Correction**

TITLE: Thienopyrimidine-based inhibitors of the Src family

DATE-ISSUED: January 7, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
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| Benish; Michele A. | Pearland | TX | N/A | N/A |
| Lawless; Michael | St. Charles | MO | N/A | N/A |
| Budde; Raymond J. A. | Bellaire | TX | N/A | N/A |

APPL-NO: 09/ 694145

DATE FILED: October 23, 2000

US-CL-CURRENT: 514/260.1, 544/278

ABSTRACT:

Various thienopyrimidine-based analog compounds that selectively inhibit the Src family of tyrosine kinases. These compounds are thienopyrimidines and contain a hydrozone bridge created by heating a thienopyrimidine hydrazine with an aldehyde in ethanol at reflux. Such compounds are useful in the treatment of various diseases including hyperproliferative diseases, hematologic diseases, osteoporosis, neurological diseases, autoimmune diseases, allergic/immunological diseases, or viral infections.

99 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

---------- KWIC ---------

Brief Summary Text - BSTX (12):
Herpesviridae, papovaviridae, and retroviridae have been shown to interact with non-receptor tyrosine kinases and use them as signaling intermediates. The HIV-1 Nef protein interacts with members of the Src family of tyrosine kinases. Nef mediates downregulation of CD4 membrane expression, modification of T-cell activation pathways, and increases virus infectivity (Collette et al., 1997). The HBx protein of the hepatitis B virus is essential for infection by hepadnaviruses and activates Ras by activating the Src family of tyrosine kinases. The activation of Ras is necessary for the ability of the HBx protein to stimulate transcription and release growth arrest in quiescent cells (Klein and Schneider, 1997). Activity of the Src family of tyrosine kinases is altered by association with viral proteins such as mouse and hamster polyomavirus middle-T antigens, Epstein-Barr virus LMP2A, and herpesvirus saimiri Tip (Dunant and Ballmer-Hofer, 1997).

Detailed Description Text - DETX (255):
Klein N P and Schneider R J. Activation of Src Family Kinases by Hepatitis B

Virus HBx Protein and Coupled Signaling to Ras. Mol Cell Biol 17:6427-6436, 1997.

US-PAT-NO: 6420338

DOCUMENT-IDENTIFIER: US 6420338 B1

TITLE: Inhibition of the Src kinase family pathway as a method of treating HBV infection and hepatocellular carcinoma

DATE-ISSUED: July 16, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Schneider; Robert J. | New York | NY | N/A | N/A |
| Klein; Nicola | New York | NY | N/A | N/A |


APPL-NO: 08/ 874430

DATE FILED: June 13, 1997

US-CL-CURRENT: 514/12, 514/262.1 , 514/451 , 514/619 , 514/646 , 514/789

ABSTRACT:

The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target HBx mediated activation of Src kinase, members of the Src tyrosine kinase family and components of the Src kinase family signal transduction pathways for the treatment of HBV infection and related disorders and diseases, such as HCC. The invention further relates to pharmaceutical compositions for the treatment of HBV infection targeted to HBx and its essential activities required to sustain HBV replication. The invention is based, in part, on the Applicants' discovery that activation of Src kinase signaling cascades play a fundamental role in mammalian hepadnavirus replication. Applicants have demonstrated that HBx mediates activation of the Src family of kinases and that this activation is a critical function provided by HBx for mammalian hepadnavirus replication.

9 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

---------- KWIC ---------

Abstract Text - ABTX (1):

The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target HBx mediated activation of Src kinase, members of the Src tyrosine kinase family and components of the Src kinase family signal transduction pathways for the treatment of HBV infection and related disorders and diseases, such as HCC. The invention further relates to pharmaceutical compositions for the treatment of HBV infection targeted to HBx and its essential activities required to sustain HBV replication. The invention is based, in part, on the Applicants' discovery that activation of Src kinase signaling cascades play a fundamental role in mammalian hepadnavirus replication. Applicants have demonstrated that HBx mediates activation of the Src family of kinases and that this activation is a critical function provided by HBx for mammalian hepadnavirus replication.

TITLE - TI (1):
Inhibition of the Src kinase family pathway as a method of treating HBV infection and hepatocellular carcinoma

Brief Summary Text - BSTX (2):
The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target Src family kinases and components of the Src kinase family signal transduction pathways, including HBx activation of Src kinase family signal transduction pathways for the treatment and prevention of hepatitis B virus (HBV) infection and hepatocellular carcinoma (HCC). The invention also relates to screening assays to identify potential antiviral agents which target HBx-mediated activation of Src kinase signaling cascades for the treatment of HBV.

Brief Summary Text - BSTX (17):
The present invention relates to the treatment and prevention of HBV infection by targeting activation of the Src family of kinases. The present invention also relates to compounds which inhibit HBx-mediated activation of the Src family of kinases as well as the downstream components of the Src kinase signaling cascade for the treatment of HBV infection.

Brief Summary Text - BSTX (18):
The invention is based, in part on the Applicants' surprising discovery that activation of a Src kinase signaling cascade is a critical function provided by HBx for mammalian hepadnavirus replication. The Applicants have shown that Src kinases are also activated during HBV infection of cultured cells and that this activation is an essential function of the viral HBx protein. Thus, the Applicants have demonstrated that the HBx-mediated activation of the Src kinase signaling cascade plays a fundamental role in mammalian hepadnavirus replication.

Brief Summary Text - BSTX (19):
The Applicants have demonstrated that HBx mediated activation of Src kinase signaling cascade is an effective target for HBV anti-viral agents since activation of this pathway is essential for HBV replication. Therefore, targeting HBx for the treatment of HBV should result in a highly specific and efficacious method of blocking HBV replication. The Src family of kinases, although host cell gene products, are only activated in proliferating or differentiating cells, and in cells infected by many DNA and tumor viruses. Therefore, targeting the Src family of kinases for the treatment of HBV infection should result in a therapeutic with a high degree of efficacy and sufficient specificity with side effects no more toxic than chemotherapeutics currently used to treat cancer.

Brief Summary Text - BSTX (20):
The present invention encompasses a variety of techniques and compounds to target the activities of HBx essential for HBV replication. In particular, these include, but are not limited to HBx-mediated activation of the Src kinase family signal transduction pathways for the treatment and prevention of HBV infection. The invention encompasses the use of known Src inhibitors to treat HBV infection. Examples of such specific inhibitors include, but not limited to: Src specific tyrosine kinase inhibitors, such as CsK, tyrphostin-derived inhibitors, derivatives of benzylidenemalonitrile, pyrazolopyrimidine PP1, and microbial agents, such as angelmicin B; and competitive inhibitors, such as small phosphotyrosine containing ligands. The invention also encompasses the use of known HBx inhibitors for the treatment of HBV, including, but not limited to, antisense RNAs directed to HBx. The present invention also relates to the use of inhibitors of downstream effectors of Src kinases, including but

not limited to, cytoplasmic factors, such as Ras, Raf, focal adhesion kinase (FAK) and MAPK, and nuclear factors, such as Myc and cyclin-dependent kinases.

Brief Summary Text - BSTX (21):
In another embodiment of the present invention gene therapy approaches, including dominant-negative mutants, antisense molecules and SELEX RNAs targeted to block Src kinase or HBx gene expression, may be used as a method to treat and prevent HBV infection and HCC. In yet another embodiment of the invention, upstream and downstream components and effectors of the Src kinase family signaling cascade may be targeted by gene therapy approaches to inhibit HBV infection.

Brief Summary Text - BSTX (22):
The present invention further relates to screening assays to identify compounds which inhibit. HBx-mediated activation of the Src kinase signaling pathway and may be used to treat HBV infection and diseases and disorders associated with HBV infection.

Brief Summary Text - BSTX (23):
The invention is illustrated by way of working examples which demonstrate that HBx mediates activation of a Src kinase signaling cascade and that activation of this signaling cascade is an essential function of HBx required to sustain HBV replication. The working examples of the present invention further demonstrate the ability of inhibitors of the Src kinase signaling cascade to inhibit HBV replication.

Brief Summary Text - BSTX (26):
As used herein, the term "target protein" refers to those proteins which correspond to Src kinase or members of the Src kinase family or components of the Src kinase signaling pathway or proteins encoded by the HBV genome, including HBx.

Drawing Description Text - DRTX (7):
FIG. 6. Woodchuck Hepatitis B Virus (WHV) HBx protein (WHx) activates a Src-Ras signaling cascade during WHV replication in cultured cells. Chang cells were co-transfected with 20 .mu.g pcWHV or wtWHV with 8 .mu.g of either dominant-negative Ras, kinase inactive (dominant-negative) Src, or Csk plasmids. Eighteen hours post-transfection, cells were serum-starved in 0.5% CS for 20 hours, MAP kinase (ERK-2) was immunoprecipitated from equal amounts of cell lysates and pellets were subjected to an in vitro MBP kinase assay.

Drawing Description Text - DRTX (8):
FIG. 7. WHx requires activation of Src family kinase for WHV replication. Chang cells were co-transfected with 20 .mu.g PCWHV, wtWHV, wtWHV and RasDN (dominant-negative) (10 .mu.g), or wtWHV and Csk (10 .mu.g). Three days post-transfection viral core-associated DNA was isolated, purified, and subjected to Southern blot analysis using a full-length .sup.32 P-labeled WHV genomic probe.

Detailed Description Text - DETX (2):
The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target HBx-mediated activation of Src kinase, members of the Src tyrosine kinase family and components of the Src kinase family signal transduction pathways for the treatment of HBV infection and related disorders and diseases, such as HCC. The invention further relates to pharmaceutical compositions for the treatment of HBV-infection targeted to HBx and its essential activities required to sustain HBV replication.

Detailed Description Text - DETX (3):

The invention is based, in part, on the Applicants' discovery that activation of Src kinase signaling cascades plays a fundamental role in mammalian hepadnavirus replication. Applicants have demonstrated that HBx mediates activation of the Src family of kinases and that this activation is a critical function provided by HBx for mammalian hepadnavirus replication.

Detailed Description Text - DETX (6):

The present invention relates to cell-based and animal model based screening assays to identify novel anti-HBV agents which target HBx and its interaction and/or activation of cellular components of the Src kinase signaling cascade. In addition, the present invention relates to screening assays to identify novel antiviral agents which inhibit HBx mediated activation of Src kinase and/or downstream effectors of the Src kinase signaling cascade, such as the nuclear factor, Myc. A variety of protocols and techniques may be utilized to screen for agents which interfere with and/or inhibit the interaction and/or activation of the Src kinase signaling cascade by HBX.

Detailed Description Text - DETX (9):

5.1 The Role of HBx Mediated SRC Kinase Activation in HBV-Infection and its Use as a Target for Intervention

Detailed Description Text - DETX (10):

The present invention is based, in part, on the Applicants' surprising discovery that (1) HBx acts as an intracellular, cytoplasmic activator of the Src family of nonreceptor tyrosine kinases; (2) HBx stimulates tyrosine kinase activity of the Src family kinase members, including c-Src and c-Fyn; and (3) inhibition of Src activity by the expression of a Src inhibitor, e.g., the Csk protein, results in the dramatic inhibition of HBV replication. This discovery is exemplified in the in Sections 6, 7, 8 and 9 infra, which demonstrate that activation of Src kinase and the Src kinase signaling cascade is required to sustain HBV replication, and that inhibition of Src kinase dramatically inhibits HBV replication.

Detailed Description Text - DETX (14):

Applicants have further demonstrated that the expression a Src inhibitors, i.e., Csk protein, or dominant-negative Src or Fyn proteins resulted in the inhibition of HBx activation of downstream components of Src kinase signaling cascade. Applicants have also shown that the expression of Src dominant-negative mutants, such as Csk, inhibited the ability of HBx to stimulate activities of the nuclear factor, Myc, including stimulation of cell cycle progression by blocking HBx activation of Src kinase signaling pathways. These findings clearly establish that activation of a Src kinase signaling cascade by HBx has a critical role in the hepadnaviral life cycle.

Detailed Description Text - DETX (15):

HBx mediated activation of Src is required for HBV replication as demonstrated by way of example (Section 9 infra). The Applicants' work demonstrates that an essential component of the requirement of HBx viral replication in cultured cells is its ability to activate Src signaling cascades. HBx activation of a Src signaling cascade plays a critical role in transcriptional upregulation of the viral mRNAs. Inhibition of Src activity by the expression of a Src inhibitor, e.g., the CsK protein, results in the dramatic inhibition of HBV replication. These results illustrate that activation of Src family kinases has an essential role during HBV replicative life cycles.

Detailed Description Text - DETX (16):

The Applicants' discovery has implicated several targets for effective HBV anti-viral agents. HBV therapies that target the viral gene product HBx should

result in a high degree of specificity and efficacy. HBV therapies that target the host cell gene products, the Src family of kinases, should likewise demonstrate specificity and efficacy. Although host cell gene products, the Src family of kinases are active in proliferating cells, such as cancer cells, or in virally infected cells. Therefore, targeting the Src family of kinases for the treatment of HBV infection should result in a high degree of efficacy, and sufficient specificity with side effects no more toxic than chemotherapeutics currently used to treat cancer.

Detailed Description Text - DETX (17):
5.2 Treatment of HBV-Infection Using Inhibitors of HBx Medicated Src Activation

Detailed Description Text - DETX (18):
The present invention encompasses a variety of therapeutic protocols, methods and compounds to target HBx-mediated activation of the Src kinase signaling cascade for the treatment of HBV. The present invention encompasses all of the compounds described in the subsections below to target HBx-mediated activation of the Src kinase signaling cascade with the proviso that they are not known in the art to be used to treat HBV infection, including, for example, interferon .alpha., interferon .delta., interleukin-1, interleukin-2, immune-active peptides, such as thymosin-alpha, nucleoside analogs, such as vidarabine, fialuridine, lamivuridine, famciclovir, ribavarin, and corticosteroids, such as prednisone and azathioprine.

Detailed Description Text - DETX (23):
A variety of techniques and compositions may be utilized to target Src kinase to inhibit its activity or to inhibit HBx mediated activation of components of the Src kinase mediated signaling cascade, thereby inhibiting HBV replication. Such techniques and compositions may include, but are not limited to, gene therapy approaches, drugs, small organic molecules identified to inhibit Src kinase, Ras, Raf, MAPK kinase, MAPK, c-Myc, cyclin-dependent kinases and/or other downstream effectors of the Src kinase signaling cascade.

Detailed Description Text - DETX (24):
In particular, compounds which may be used in accordance with the present invention to specifically target activation of Src kinase are binding proteins and competing ligands that prevent the intramolecular interaction between the carboxy-terminal phosphorylated tyrosine residue and the SH2 domain located in the amino-terminal half of the molecule and the immediately adjacent SH3 domain (Lin et al., 1993, Oncogene 8:1119-1126). In particular, compounds which may also be used in accordance with the present invention include tyrosine kinase inhibitors which block the activity the Src kinase signaling cascade and therefore would block HBV replication. Examples of such tyrosine kinase inhibitors include, but are not limited to, tyrphostin-derived inhibitors, which are derivatives of benzylidenemalonitrile, have been shown to have strong inhibitory activity of Src (Ramdas et al., 1995, Archives of Biochemistry and Biophysics 323:237-242), pyrazolopyrimidine PP1 (4-amino-5-(4-methylphenyl)-7-(t-butyl) pyrazolo [3,4-d] pyrimidine, a selective inhibitor of the Src family of kinases (Hanke et al., 1996, J. Biol. Chem. 271:695-791) and derivatives thereof. Other examples include microbial agents, such as angelmicin B, a specific inhibitor of Src tyrosine kinase activity, and derivatives thereof (Yokoyama et al., 1996, Leukemia Research 20:491-497), which may also be used to inhibit HBV replication.

Detailed Description Text - DETX (25):
In another embodiment of the present invention, small peptides which compete with larger phosphotyrosine peptides for binding to the Src kinase protein may be used to inhibit the Src kinase signaling cascade, in particular small

phosphotyrosine containing peptide ligands, 5 to 6 amino acids, which are able to compete with larger phosphotyrosine-containing peptides and protein ligands for binding to SH2 domains, thereby inhibiting the Src kinase signaling cascade and blocking replication of HBV. Another embodiment of the present invention includes small peptides which correspond to catalytic or enzymatic domains of Src kinase and would compete with Src kinase, inhibiting the activation of downstream components of the Src kinase signaling cascade. Another embodiment includes the use of larger polypeptides that inhibit Src kinase activity including, but not limited to, Csk (carboxyl-terminal Src kinase) which is a specific physiologic inhibitor of Src kinase. Further examples of larger polypeptides that inhibit Src kinase activity include, for example, Src dominant-negative mutants, i.e., Srck-(Barone et al., 1995, Nature 378:509-512) and Fyn dominant-negative mutants (Twamley-Stein et al., 1993, Proc. Natl. Acad. Sci. USA 90:7696-7700), also included are dominant-negative mutants of downstream effectors of the Src kinase signaling cascade, including Ras, Raf, MAPK kinase, MAPK dominant-negative mutants and Myc dominant-negative mutants (Sawyers et al., 1992, Cell 70:901-910).

Detailed Description Text - DETX (28):
In one embodiment of the invention, novel antiviral agents identified by the screening methods of the present invention are used in combination with known therapies to treat HBV infection, for example, IFN, interleukin-1, interleukin-2, immune-active peptides, nucleoside analogs and corticosteriods. The antiviral agents identified by the screening methods of the present invention may also be used in combination with exogenous or endogenous agents which induce IFN expression. In yet another embodiment, inhibitors of Src kinase are used in combination with agents which induce an anti-HBV immune response in order to target two different molecules required in the viral life cycle.

Detailed Description Text - DETX (47):
In yet another specific embodiment, attenuated viruses, such as hepadnaviruses, which have the same tropism as HBV, may be engineered and used for gene therapy in accordance with the present invention. Hepadnaviruses are particularly attractive for use in gene therapy in accordance with the present invention as these viruses will deliver the therapeutic exactly to those cells infected with HBV. Hepadnaviral vectors would be particularly effective for the delivery of nucleic acids targeting components of the Src kinase signaling cascade, thereby avoiding unnecessarily knocking out expression of host genes.

Detailed Description Text - DETX (89):
At least two different assay systems, described in the subsections below, can be designed and used to identify compounds or compositions that modulate HBx-mediated activation of Src kinase signaling cascades and thereby inhibit HBV replication.

Detailed Description Text - DETX (90):
The systems described below may be formulated into kits. To this end, cells expressing HBx and components of the Src kinase signaling cascade, or cells expressing components of the Src kinase signaling cascade which are capable of sustaining HBV replication, or cell lysates thereof can be packaged in a variety of containers, e.g., vials, tubes, microtitre well plates, bottles, and the like. Other reagents can be included in separate containers and provided with the kit; e.g., positive control samples, negative control samples, buffers, cell culture media, etc.

Detailed Description Text - DETX (94):
Alternately, cell lines which co-express HBx and Src kinase and components of the Src kinase signaling cascade may be genetically engineered to assay for

inhibitors of HBx activation of Src. This can be engineered in cell in the absence of HBV replication or in cell lines which support the HBV life cycle as a means of (1) identifying additional factors required to support the HBV life cycle, and (2) as a system to screen test compounds, for their ability to interfere with HBx activation and/or interaction with the Src kinase, and (3) as a system to screen test compounds for their ability to inhibit Src kinase activity and therefore inhibit HBV replication.

Detailed Description Text - DETX (103):
Alternatively, activation of Src kinase signaling pathways mediated by HBx may be measured by the secretion of mature HBV viral particles into the medium of growing Chang cells. For example, Chang liver cells may be stably transformed with an HBV or WHV pregenome, or with a head-to-tail dimer of either HBV or WHV genomes. The integrated virus will produce and secrete HBV/WHV particles into the medium. As demonstrated by the Applicants, the secretion of viral particles is strongly enhanced by HBx protein activation of Src kinases. If the test compound is effective in inhibiting HBx activation of Src, it will result in reduced secretion of HBV/WHV particles into the medium. The level of particle secreted into the medium can be assayed using commercial ELISA kits to detect the presence of HBV/WHV HBcAg and HBsAg.

Detailed Description Text - DETX (109):
In preferred embodiment of the invention, Src kinase is expressed alone in transgenic Src knock-out mice and a HBV pseudovirus is used to infect the animals. For example, a HBV pseudovirus which contains the HBV virus and an envelope protein from a virus with a natural tropism for murine cells, such as the murine leukemia virus (MLV), is used to bypass internalization of the HBV virus by the murine cells. These murine cells can then support the life cycle of the internalized HBV virus, because they express human Src kinase.

Detailed Description Text - DETX (111):
In yet another embodiment of the animal model screens of the present invention, the effect of test compounds to inhibit HBV-replication may be measured indirectly. For example, transgenic mice may be engineered which express (1) the HBx gene product under the control of an inducible promoter, and (2) readout vector which is responsive to Src activation. The readout vector may comprise a reporter gene under the control of a Myc promoter. Such reporter constructs are described in Section 5.5.1 infra. In this assay system, expression of the HBx gene product is induced and the test compound is administered to the mice. The ability of the test compound to inhibit HBx mediated activation of Src kinase and HBV replication is assayed by measuring the reporter gene. Such reporter genes may include but are not limited to chloramphenicol acetyltransferase (CAT), luciferase, GUS, growth hormone, or placental alkaline phosphatase (SEAP). Following exposure of the animal to the test compound, the level of reporter gene expression may be quantitated from the blood or tissue sample to determine the test compound's ability to regulate receptor activity. Alkaline phosphatase assays are particularly useful in the practice of the invention as the enzyme is secreted from the cell. Therefore, tissue culture supernatant may be assayed for secreted alkaline phosphatase. In addition, alkaline phosphatase activity may be measured by calorimetric, bioluminescent or chemilumenscent assays such as those described in Bronstein, I. et al. (1994, Biotechniques 17: 172-177). Such assays provide a simple, sensitive easily detection system for pharmaceutical screening.

Detailed Description Text - DETX (166):
WHV Requires SRC Family Kinases for in vitro Replication

Detailed Description Text - DETX (171):
Chang cells were co-transfected with plasmids encoding either wtWHV, pCWHV,

wtWHV and RasDN, or wtWHV and Csk, intracellular core-associated viral DNA was isolated 3 days post-transfection, and purified viral DNA analyzed by Southern blot hybridization as described above (FIG. 7). Consistent with the previous data, accumulation of viral DNA replicative intermediates was strongly enhanced in cells expressing wtWHV, as compared to cells expressing pCWHV. Co-expression of RasDN protein with wtWHV had no detectable effect on viral replication, and viral DNA was synthesized at near wild-type levels. Expression of the RasDN protein impaired WHV activation of MAP kinase under these same experimental conditions (FIG. 7), indicating that the RasDN protein was able to function during WHV replication. However, these results illustrate the effects of one particular inhibitor of Ras and do not provide an explanation of the mechanism by which activation of Src kinase supports HBV replication. In sharp contrast, co-expression of Csk with wtWHV completely abolished the ability of wtWHV to replicate to high levels. These results demonstrated that an essential component of the requirement of HBx during in vitro viral replication in Chang cells is its ability to activate Src signaling cascades, and that activation of Src family kinases has a critical role during the viral replicative life cycle.

Detailed Description Text - DETX (173):

To assess whether HBx activation of a Src signaling cascade plays an essential role in transcriptional upregulation of the viral mRNAs, Chang cells were co-transfected with wtWHV and either RasDN or Csk plasmids, and the RNA visualized by Northern analysis (FIG. 7, lanes 2 and 3). Co-expression of RasDN with wtWHV only slightly reduced the amount of the RNA species, while co-expression of wtWHV with Csk reduced the RNA level .about.3-5 fold, to the level also observed by expression of PCWHV. To ensure that the decrease in synthesis of WHV RNA by Csk was not the unforseen consequence of Csk inhibition of the CMV promoter (which drives synthesis of the WHV pregenomic RNA), control experiments assessing the effect of Csk on a CMV-.beta.gal reporter were carried out. In comparison with expression of CMV-.beta.gal alone, co-expression of Csk with CMV-.beta.gal did not significantly alter expression of the .beta.gal protein as measured by its .beta.-galactosidase activity (Sambrook et al, 1989, supra). This control experiment indicates that expression of Csk does not generally inhibit transcription of the CMV promoter, and rules out a non-specific effect of Csk on viral transcription. Therefore, these data suggest that the HBx protein moderately increases the abundance of all the viral transcripts through activation of the Src family of kinases. However, the WHx-induced increase in mRNA abundance (.about.3-5 fold) is much less then the HBx-induced increase in viral DNA synthesis (.about.20-30 fold). Therefore, transcriptional transactivation by HBx does not appear to fully account for the augmentation of viral replication by the HBx protein. The stimulation of Src signaling cascades by HBx must therefore promote WHV replication independent of the effect of WHx on viral transcription. These results illustrate that HBx activates a Src-Ras signaling cascade during viral replication in vitro which is essential for the host cell to sustain HBV replication.

Detailed Description Text - DETX (175):

WHV Requires SRC Family of Kinases for in vitro Replication

Detailed Description Text - DETX (176):

The requirement for activation of Src kinase family members in replication of WHV can be determined in woodchuck infected livers in the following manner. A 2-5 year old woodchuck is experimentally infected using a pooled serum from previous chronic carrier woodchucks. After 2 years of chronic infection, determined by WHsAg ELISA, the infected liver is surgically removed, the liver is perfused as described (Jacob et al., 1994, Exp. Cell Res. 212:42-48), hepatocytes are dispersed by collagenase treatment and plated onto collagen

coated dishes in L15 medium supplemented with 5% fetal calf serum, hydrocortisone and insulin. To introduce an inhibitor of Src kinases into primary hepatocytes, the Csk gene is cloned into the left-end of a replication-defective adenovirus vector under the control of the CMV promoter, as described (Doria et al., 1995, EMBO J. 14:4747-4757). Adenovirus vectors infect primary cells and express trans-genes efficiently, whereas it is not possible to transfect such cells at a high rate. Within several days of plating, cells are infected with the Csk-adenovirus vector, medium is replaced with L15 medium lacking insulin and containing reduced serum (between 0.5-2%). Cells are then harvested at 2 and 4 days after introduction of the vector. The medium can be assayed for levels of secreted WHV by ELISA for WHcAg and WHsAg. The level of virus replication can be assayed as described for Chang cells.

Claims Text - CLTX (1):

1. A method for treating Hepatitis B virus (HBV) infection or inhibiting HBV virus replication comprising administering a compound to an HBV-infected patient that decreases the activity of Src kinase.

Claims Text - CLTX (2):

2. A method for inhibiting Hepatitis B virus (HBV) infection or replication, comprising administering a Csk protein to decrease the activity of Src kinase in a subject.

US-PAT-NO: 6355248

DOCUMENT-IDENTIFIER: US 6355248 B1
**See image for Certificate of Correction**

TITLE: Method of modulating an immune response in an infected mammal by transmucosal administration of modulating agent

DATE-ISSUED: March 12, 2002


INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
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| Block; Timothy | Doylestown | PA | N/A | N/A |



APPL-NO: 09/ 334819

DATE FILED: June 17, 1999


PARENT-CASE:

This application is a continuation of copending International application PCT/US98/04116 filed on Jan. 2, 1998 and published in English under PCT Article 21(2) on Jul. 9, 1998 publication no. WO 98/29121), which claims the benefit of U.S. provisional application Ser. No. 60/034,596, filed Jan. 2, 1997.


US-CL-CURRENT: 424/189.1, 424/193.1 , 424/227.1 , 514/49



ABSTRACT:

Methods and compositions for modulating an immune response in a mammal infected with a bacterium, a virus, or a parasite are provided. The methods and compositions are useful in mammals experiencing an acute infection and in mammals experiencing a chronic infection. The methods and compositions may be used in conjunction with a known treatment for infection of a mammal by an infectious agent. Methods and compositions for transmucosal delivery of a molecule comprising an epitope located in close proximity to the immune response are provided.


27 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

---------- KWIC ---------

Detailed Description Paragraph Table - DETL (1):
TABLE 1 Detection of stimulated CTL by detection of label release from cultured spleen cells. ".gamma.-IFN" means gamma-interferon. "E:T" means the ration of effector cells to target cells. Effector cells were .gamma.-IFN Percent.sup.51 Cr released collected from mice stim- E:T E:T E:T E:T immunized with: Target cells ulated? 1:100 1:33 1:11 1:4 MLE-10/HBV MLE-10 no 7 5 3 3 MLE-10/HBV MLE-10/ no 13 10 9 7 HBV MLE-10/**HBV MLE-10 yes** 7 6 6 2 MLE-10/**HBV MLE-10/ yes** 25 23 19 15 HBV

US-PAT-NO: 6274788

DOCUMENT-IDENTIFIER: US 6274788 B1

TITLE: Bicistronic DNA construct comprising X-myc transgene for use in production of transgenic animal model systems for human hepatocellular carcinoma and transgenic animal model systems so produced

DATE-ISSUED: August 14, 2001


INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Kumar; Vjay | New Delhi | N/A | N/A | IN |
| Singh; Mahavir | New Delhi | N/A | N/A | IN |
| Totey; Satish | New Delhi | N/A | N/A | IN |
| Anand; Rajesh | New Delhi | N/A | N/A | IN |



APPL-NO: 09/ 243282

DATE FILED: February 2, 1999

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---|---|---|
| IN | 2858/98 | September 23, 1998 |

US-CL-CURRENT: 800/18, 536/23.5 , 536/24.33 , 800/3 , 800/8



ABSTRACT:

The present invention relates to a bicistronic DNA construct comprising X-myc transgene. In particular, the present invention relates to a bicistronic X15-myc transgene capable of expressing truncated X protein and a full-length murine c-myc protein. More particularly, the present invention relates to a bicistronic DNA construct being an X15-myc transgene for use in the production of transgenic animal model systems for human hepatocellular carcinoma and transgenic animal model systems so produced. The invention is based partially on the discovery that in susceptible transgenic mice that carry a bicistronic X-myc transgene there is an accelerated formation of liver tumors involving all lobes.


5 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

---------- KWIC ---------


Other Reference Publication - OREF (14):

Klein, N., et al. "Activation of Src Family Kinases by Hepatitis B Virus HBx Protein and Coupled Signaling to Ras." Molecular and Cellular Biology, vol. 17, No. 11 (Nov. 1997) pp. 6427-6436.

US-PAT-NO: 5888779

DOCUMENT-IDENTIFIER: US 5888779 A

TITLE: Kits for nucleic acid sequence amplification methods

DATE-ISSUED: March 30, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Kacian; Daniel Louis | San Diego | CA | N/A | N/A |
| Fultz; Timothy J. | Vista | CA | N/A | N/A |


APPL-NO: 08/ 461654

DATE FILED: June 5, 1995

PARENT-CASE:

This application is a continuation of U.S. Ser. No. 07/550,837, filed Jul. 10, 1990, issued as U.S. Pat. No. 5,480,784, which is a continuation-in-part of U.S. Ser. No. 07/379,501, filed Jul. 11, 1989, abandoned.

US-CL-CURRENT: 435/91.2, 435/91.21 , 435/975

ABSTRACT:

Kits for synthesizing multiple copies of a target nucleic acid sequence autocatalytically under conditions of substantially constant temperature, ionic strength, and pH are provided in which multiple RNA copies of the target sequence autocatalytically generate additional copies. These methods are useful for generating copies of a nucleic acid target sequence for purposes which include assays to quantitate specific nucleic acid sequences in clinical, environmental, forensic and similar samples, cloning and generating probes.

16 Claims, 28 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 26

---------- KWIC ---------

Detailed Description Paragraph Table - DETL (5):

TABLE 5 _____ Preliminary Procedure II Reaction Kinetics. Reaction 1 2 3 4 5 6 7 _____ Target No Yes Yes Yes Yes Yes Yes (10 amol) T7pro(+) Yes No Yes No No Yes Yes T7pro(-) Yes No No Yes No No Yes HBV(-)Pr Yes No No No Yes Yes No Time (minutes) Minus Product (RLU's) 0 619 638 635 703 592 619 656 30 613 635 613 755 626 644 1133 60 635 649 856 894 635 2146 6008 90 593 619 619 925 624 6226 23484 120 621 606 627 946 639 12573 43939 180 678 635 714 930 627 21719 78682 Plus Product (RLU's) 0 624 646 1661 710 621 636 962 30 637 601 802 629 655 803 758 60 639 706 800 679 664 226 2895 90 638 683 956 633 687 7786 8085 120 643 670 884 647 632 18160 18241 180 683 617 968 758 712 34412 41165 _____

US-PAT-NO: 5824518

DOCUMENT-IDENTIFIER: US 5824518 A

TITLE: Nucleic acid sequence amplification methods

DATE-ISSUED: October 20, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Kacian; Daniel Louis | San Diego | CA | N/A | N/A |
| Fultz; Timothy J. | Vista | CA | N/A | N/A |


APPL-NO: 08/ 469067

DATE FILED: June 6, 1995

PARENT-CASE:

This a continuation of Kacian et al., U.S. Ser. No. 07/550,837, filed Jul. 10, 1990, now U.S. Pat. No. 5,480,784, hereby incorporated by reference herein in its entirety (including the drawings) which is a CIP of U.S. Ser. No. 07/379,501, filed Jul. 11,1989, now abandoned.

US-CL-CURRENT: 435/91.21

ABSTRACT:

Methods of synthesizing multiple copies of a target nucleic acid sequence autocatalytically under conditions of substantially constant temperature, ionic strength, and pH are provided in which multiple RNA copies of the target sequence autocatalytically generate additional copies. These methods are useful for generating copies of a nucleic acid target sequence for purposes which include assays to quantitate specific nucleic acid sequences in clinical, environmental, forensic and similar samples, cloning and generating probes.

14 Claims, 28 Drawing figures

Exemplary Claim Number: 10

Number of Drawing Sheets: 26

---------- KWIC ---------

Detailed Description Paragraph Table - DETL (5):

TABLE 5 _____ Preliminary Procedure. II Reaction Kinetics. _____ Reaction 1 2 3 4 5 6 7 _____ Target (10 amol) No Yes Yes Yes Yes Yes Yes T7pro(+) Yes No Yes No No Yes Yes T7pro(-) Yes No No Yes No No Yes HBV(-)Pr Yes No No No Yes Yes No Time (minutes) Minus Product (RLU's) 0 619 638 635 703 592 619 656 30 613 635 613 755 626 844 1133 60 635 649 856 894 635 2146 6008 90 593 619 619 925 624 6226 23484 120 621 606 627 946 639 12573 43939 160 678 635 714 930 627 21719 78682 Time (minutes) Plus Product (RLU's) 0 624 646 1661 710 621 636 962 30 637 601 802 629 655 803 758 60 639 706 800 679 664 226 2895 90 638 683 956 633 687 7786 8085 120 643 670 884 647 632 18160 18241 180 683 617 968 758 712 34412 41165

US-PAT-NO: 5480784

DOCUMENT-IDENTIFIER: US 5480784 A
**See image for Certificate of Correction**

TITLE: Nucleic acid sequence amplification methods

DATE-ISSUED: January 2, 1996

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Kacian; Daniel L. | San Diego | CA | N/A | N/A |
| Fultz; Timothy J. | Vista | CA | N/A | N/A |


APPL-NO: 07/ 550837

DATE FILED: July 10, 1990

PARENT-CASE:

This application is a continuation-in-part of App. Ser. No. 379,501 filed Jul. 11, 1989 now abandoned.

US-CL-CURRENT: 435/91.21, 435/91.2

ABSTRACT:

Methods of synthesizing multiple copies of a target nucleic acid sequence autocatalytically under conditions of substantially constant temperature, ionic strength, and pH are provided in which multiple RNA copies of the target sequence autocatalytically generate additional copies. Nucleotide sequences of target nucleic acid portions and of primers are selected to minimize the ability of the primer to remain able to form a DNA primer extension product of for formation of an RNA:DNA primer hybrid and exposure to RNase H.

6 Claims, 27 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 26

---------- KWIC ---------

Detailed Description Paragraph Table - DETL (6):
TABLE 6 _____ Preliminary Procedure II System. Reaction 1 2 3 4 5 6 7 _____ M13L(-) No No Yes Yes Yes Yes Yes T7pro(+) Yes Yes No Yes No Yes Yes T7pro(-) Yes No No No Yes Yes No HBV(-)Pr No Yes No No No No Yes _____ Probe Relative Light Units (RLU's) Probe(+) 862 744 762 1089 2577 96221 30501 Probe(-) 473 420 483 3038 1080 15171 14863 _____

US-PAT-NO: 5399491

DOCUMENT-IDENTIFIER: US 5399491 A

TITLE: Nucleic acid sequence amplification methods

DATE-ISSUED: March 21, 1995

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Kacian; Daniel L. | San Diego | CA | N/A | N/A |
| Fultz; Timothy J. | Vista | CA | N/A | N/A |


APPL-NO: 07/ 855732

DATE FILED: March 19, 1992

PARENT-CASE:

This is a continuation of application Ser. No. 07/550,837, filed Jul. 10, 1990, which is a continuation-in-part of app. Ser. No. 379,501, filed Jul. 11, 1989, abandoned.

US-CL-CURRENT: 435/91.21, 435/6 , 435/91.2 , 536/24.33

ABSTRACT:

Methods of synthesizing multiple copies of a target nucleic acid sequence autocatalytically under conditions of substantially constant temperature, ionic strength, and pH are provided in which multiple RNA copies of the target sequence autocatalytically generate additional copies. These methods are useful for generating copies of a nucleic acid target sequence for purposes which include assays to quantitate specific nucleic acid sequences in clinical, environmental, forensic and similar samples, cloning and generating probes.

49 Claims, 28 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 26

---------- KWIC ---------

Detailed Description Paragraph Table - DETL (5):

TABLE 5 _____ Preliminary Procedure II Reaction Kinetics. Reaction 1 2 3 4 5 6 7 _____ Target No Yes Yes Yes Yes Yes Yes (10 amol) T7pro(+) Yes No Yes No No Yes Yes T7pro(-) Yes No No Yes No No Yes HBV(-)Pr Yes No No No Yes Yes No Time (minutes) Minus Product (RLU's) 0 619 638 635 703 592 619 656 30 613 635 613 755 626 844 1133 60 635 649 856 894 635 2146 6008 90 593 619 619 925 624 6226 23484 120 621 606 627 946 639 12573 43939 180 678 635 714 930 627 21719 78682 Plus Product (RLU's) 0 624 646 1661 710 621 636 962 30 637 601 802 629 655 803 758 60 639 706 800 679 664 226 2895 90 638 683 956 633 687 7786 8085 120 643 670 884 647 632 18160 18241 180 683 617 968 758 712 34412 41165 _____